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RELIABILITY AND SIGNIFICANCE OF THE GORDON TEST IN HODGKIN'S DISEASE

PAUL E. STEINER, M.D.

CHICAGO

In 1932 Gordon described the reaction of animals to lymphogranulomatous tissues, forming the basis for a test which has since come to bear his name.[1] Twort [2] earlier observed that the subcutaneous injection of such tissues into the guinea pig produced a transitory inflammatory nodule which was sterile on all examinations. Gordon described a syndrome consisting of spastic paralysis, incoordination and ataxia, retraction of the head, fits and loss of weight which was produced in rabbits and guinea pigs on intracerebral injection of a broth suspension of diseased lymph nodes. His studies aroused wide interest because they suggested new clues as to the possible cause of the disease and also because they provided a biologic laboratory test for its diagnosis. While the view at one time propounded by Gordon that the agent responsible for this reaction is a virus [3] has not been substantiated,[4] the value of the reaction in diagnosis has been widely accepted,[5] although there are dissenting opinions on this point.[6] Many workers have stressed its value in the diagnosis of atypical or difficult cases.[7]

From the Department of Pathology of the University of Chicago.

1. Gordon, M. H.: (*a*) Rose Research on Lympadenoma, Bristol, John Wright & Sons, Ltd., 1932; (*b*) Brit. M. J. **1**:641, 1933.

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3. (*a*) Gordon, M. H.: Proc. Roy. Soc. Med. **27**:1035, 1934. (*b*) Warner, E. C.: Lancet **2**:417, 1936. (*c*) Gordon, M. H.: Proc. Roy. Soc. Med. **30**:541, 1936; footnote 1.

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(Footnote continued on next page)

Two distinct problems exist in relation to the Gordon reaction. One is concerned with the reliability of the reaction in diagnosis, while the other deals with its etiologic significance. My interest in the reaction centered in the latter, and some original observations are recorded here. During these studies data also accumulated with respect to the reliability of the reaction as a test in the differential diagnosis of lymph node diseases, and this aspect is also presented. This report includes and extends the data which I published in 1938.[5p] In the brief time since 1932 a number of divergent views have been presented on some points, and these will be mentioned and harmonized if that is possible.

TECHNIC

The fresh, unfixed lymph nodes are minced with scissors and ground fine in a mortar, sand being used if the tissue is tough. Nutrient broth (p_H about 7.2) is added to make a suspension of 1 part node to 9 parts broth. Unless the original tissues and all subsequent handling were such as to insure sterility, phenol to make a concentration of 0.5 per cent is added, or the mixture may be heated to 60 C. for thirty minutes. The mixture is stored in the ice box and shaken daily. It should be tested for sterility after several days of storage in the ice box, especially if phenol was not added. The supernatant broth is used for the test after about seven days of incubation.

The test is performed on rabbits, although guinea pigs may be used. The animal is anesthetized and the top of the scalp is shaved and sterilized. From 0.4 to 0.5 cc. of the broth suspension is slowly injected into the occipital region of the cerebrum. The site selected is a silent area and is localized externally about 3 mm. lateral to the posterior third of the external occipital protuberance and about an equal distance anterior to the bony ridge or suture found in this region. The puncture can be made directly with a 22 gage hypodermic needle, without incising the skin or drilling the bone, by using a rotary motion to avoid a plug of bone in the needle, and slight pressure. A short shank (½ inch [about 1.3 cm.]) needle is used to prevent sudden deep penetration of the brain when the bone is passed. After the injection is made, the needle is permitted to rest in situ for a few minutes, until adjustments are made to the new intracranial tension, so as to avoid back leakage through the needle tract. An injection of 0.6 cc. of the suspension is now made in the ear vein according to the original technic, but this is not necessary.

After the animal recovers from the anesthetic, it should be normal and without any paralysis. A positive reaction appears after the second day, usually on the third, fourth or fifth, but sometimes later. It is characterized by spastic paralysis,

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Uhlenhuth, P., and Wurm, K.: Klin. Wchnschr. **15**:1025, 1936. (*n*) Chapman, E. M.: Ann. Int. Med. **10**:742, 1936. (*o*) Wurm, K.: Deutsches Arch. f. klin. Med. **181**:90, 1937. (*p*) Steiner, P. E.: Arch. Path. **26**:593, 1938. Gordon.[3a]

6. (*a*) Manson, M. H.: Minnesota Med. **18**:263, 1935. (*b*) Turner, J. C.; Jackson, H., Jr., and Parker, F., Jr.: Am. J. M. Sc. **195**:27, 1938. (*c*) McNaught, J. B.: J. A. M. A. **111**:1280, 1938. (*d*) King, D. P.: St. Thomas's Hosp. Rep. **3**:68, 1938.

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ataxia, incoordination, muscle weakness, tremors, opisthotonos, arching of the back, nystagmus, incontinence of urine and feces, and weight loss in various combinations. (Figure 1 illustrates one type of reaction.) Some animals must be stimulated to move before these signs are elicited. Some animals become progressively worse, with prostration and death, while others recover completely. Those animals in which the results are negative or which recover may be used again after a suitable period, as there is little sensitization and no immunity. It is advisable to use two or three animals for each test until the technic is perfected.

RELIABILITY OF THE TEST IN DIAGNOSIS

In 21 cases of Hodgkin's disease in which the diagnosis was established by microscopic examination of tissues, disinterested pathologists being consulted in every instance in which the diagnosis was not clear, the results from application of the Gordon test were as given in table 1.

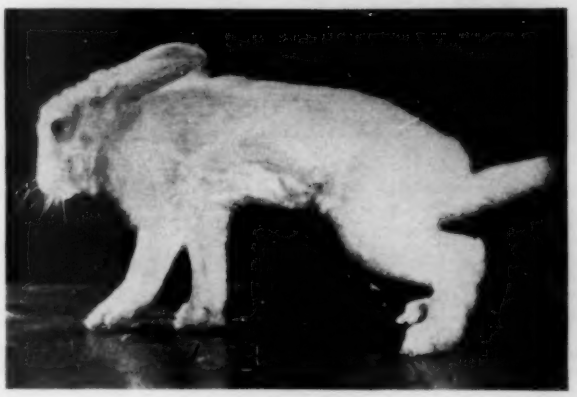

Fig. 1.—Spasticity and arched back, illustrating a positive Gordon reaction. A moment later the rabbit fell backward.

TABLE 1.—*Results from the Gordon Test in Cases of Hodgkin's Disease*

Cases tested	21
Cases in which results were positive	16
Cases in which results were negative	5
Percentage in which results were positive	76.2
Percentage in which results were negative	23.8
Total number of rabbits	59
Number of rabbits in which results were positive	30

Tissues secured at autopsy gave a greater percentage of negative results than those taken at biopsy. Thus, of 7 specimens obtained at autopsy, 3 gave negative results, while, of 12 biopsy specimens, only 2 gave negative results. In general, lymphogranulomatous lymph nodes obtained in fatal cases are more fibrous and show more necrosis than do biopsy specimens, and this has a bearing on the results. An average of nearly 3 rabbits was used in each case in which the test was made.

Only about half of them gave positive reactions. This does not present the situation fairly, because many of the negative results were obtained on retesting, with slight alteration in technic, materials which had already given negative results.

The value of any test in diagnosis depends not only on its ability to detect the disease sought but on its power to rule out confusing related diseases. A diagnostic test which detects the condition sought in only 76 per cent of the cases in which that condition is present may still have great merit if the number of false positive results is low or zero. The serologic tests used as aids in the diagnosis of late tertiary syphilis illustrate this point.

The results obtained from control tissues are therefore important. In 40 cases of lymph node disease of types which must commonly be

TABLE 2.—*Summary of Results from the Gordon Test Made with Control Tissues*

Diagnosis	Cases	Number in Which Results were Positive	Number in Which Results were Negative	Rabbits Used
Lymphosarcoma: Lymphatic	8	0	8	20
Reticulum cell	6	0	6	11
Pseudoleukemia	5	0	5	12
Leukemia: Lymphatic	6	0	6	13
Myeloid	2	0	2	6
Monocytic	2	0	2	4
Nonspecific lymphadenitis	8	0	8	18
Tuberculous lymphadenitis	3	0	3	3
Total	40	0	40	87

differentiated from Hodgkin's disease, the test was uniformly negative. The number and types of diseased lymph nodes used, with the number of experimental animals, are shown in table 2.

The test was therefore reliable in that there was no error from including cases of nonlymphogranulomatous diseases, but it did fail to detect some of the cases which on microscopic examination were called instances of Hodgkin's lymphogranuloma.

Before accepting the failure in 24 per cent of the cases at its face value as a measure of the negative error in diagnosis with the Gordon test, it is well to remember that this figure is derived by using a standard which is not without error, since all experienced histopathologists recognize that the microscopic diagnosis of lymph node diseases requires a correction factor of variable amount. Thus one has been comparing two variables. Of the control cases, those of the reticulum cell type of lymphosarcoma and some instances of nonspecific lymphadenitis offered the greatest difficulty in the differential microscopic diagnosis. All errors in differential diagnosis with regard to the former disease

cannot be eliminated, while with regard to the latter type of disease error has been reduced to a minimum by observation of the subsequent course of the patients.

The results obtained by others with lymphogranulomatous and control tissues as reported in the literature are presented in table 3. Of tissues from patients with Hodgkin's disease, about 73.9 per cent have given positive reactions and 26.1 per cent have failed to give them. My results are in this same range.

TABLE 3.—*Summary of Results with the Gordon Test, Collected from the Literature*

Author	Nodes from Patients with Hodgkin's Disease		Control Nodes	
	Positive	Negative	Positive	Negative
Gordon [8c]	75	12	..	213
van Rooyen [5d]	15	5	..	13
Ogilvie and van Rooyen [5b]	1	..	..	..
Ogilvie and van Rooyen [5c]	1	..	..	1
van der Hoeden and Huist [7a]	10	4	..	16
Dassen, R.; Fisher, A., and Fustinoni, O.: Rev. Asoc. méd. argent. **48**: 1397, 1934	1	..	..	..
Davis [5g]	11	5	..	11
Manson [6a]	9	6	3	16
Bartolozzi [5h]	3	1	..	..
Smith [5i]	2	1	..	4
Paterni and Maroncelli [5j]	2	0	2	2
Rosenberg and Bloch [5k]	2	1	..	3
Goldstein [5l]	7	2	..	20
Uhlenhuth and Wurm [5m]	5	..	..	4
Chapman [5n]	9	7	..	32
Schilling, V.: Med. Welt **11**: 167, 1937	3	0	..	..
Wurm [5o]	20	11	..	18
Turner, Jackson and Parker [6b]	5	6	..	12
d'Ovidio and Martin Vucetich [11]	1	1	..	..
Sachs and Steffel [7b]	7	1	..	5
McNaught [6c]	10	3	2	35
King [6d]	6	2	..	18
Liebegott [12b]	15	13	1±	21
Hoyt [7d]	9	0	..	..
Totals	229	81	8	444
	73.9%	26.1%	1.77%	98.23%

With control lymph nodes, however, a few workers have reported positive results. A critical analysis of these instances is undertaken in order to see how seriously these false positive results (1) interfere with the reliability of the test in practical diagnosis and (2) what they tell about the nature of the reaction.

Manson [6a] reported that 2 lymph nodes with tuberculosis and 1 showing simple hyperplasia gave positive reactions. He failed to mention the microscopic appearance of the nodes or the subsequent course of the disease. While it is true that certain hyperplastic forms of tuberculous lymphadenitis offer difficulty in differential diagnosis of lymph node diseases, the caseating forms do not. Also, the question arises as to whether these rabbits had typical positive reactions. Manson

did not give details but stated that acute reactions were seen in one to six days, and he spoke of a chronic form of reaction in which the average period of incubation is twenty-one days. Most observers of the test do not include as showing the positive reactions rabbits whose symptoms come on in one day, attributing these to traumatic injury or to meningitis by contamination, and others fail to recognize late or chronic reactions. One wonders, with McNaught,[6c] whether these delayed forms might not be due to actual tuberculous infection in the rabbit. The subsequent course of the patient whose lymph node changes were diagnosed "hyperplasia" is not stated, and the degree of histologic resemblance to lymphogranuloma is not given.

Edward [4b] stated that a lymph node with chronic myelocytic leukemia and a lymph node with myelosclerosis gave positive reactions in rabbits. Since the differentiation between these conditions and lymphogranuloma is usually not difficult on the basis of blood studies or lymph node changes, these false positive reactions do not detract from the value of the test in practical diagnosis.

McNaught [6c] described false positive results in a case of chronic hyperplastic lymphadenitis and in a case of lymphoepithelioma metastasis. The former disease might resemble lymphogranuloma and cause difficulty in diagnosis, while the latter probably would not.

Paterni and Maroncelli [5j] mentioned cases of tuberculous lymphadenitis and of chronic myelogenous leukemia which gave positive tests. The remarks made in an earlier paragraph with respect to these two conditions apply here also.

Robb-Smith [7e] saw positive reactions in a case of idiopathic eosinophilia and in one of trypanosomiasis with follicular hyperplasia. He did not state whether these caused real difficulty in differential diagnosis.

In summarizing the reported positive reactions with nonlymphogranulomatous lymph nodes it is clear that in nearly all the instances a correct differential diagnosis could easily have been made on the basis of blood counts or histologic examination.

This biologic test, then, should accompany and not replace histologic examination. It is an aid in differentiating the conditions which cause real difficulty. Robb-Smith [7e] ". . . confirms its astonishing specificity even in respect to conditions showing much histologic resemblance." In practice it may be used as follows: The suspension of lymph node in broth is prepared at the time of biopsy and placed in the ice box for autolysis. If the histologic section, prepared in the meantime, is conclusively either Hodgkin's lymphogranuloma or not, the brew can be discarded and little has been lost. If, on the other hand, the microscopic appearance is inconclusive, the biologic test can be made on the seventh day and its contribution to diagnosis secured.

SIGNIFICANCE OF THE TEST

The anatomic basis for the symptom complex produced in the animals lies in degenerative changes in the Purkinje cells of the cerebellum. These are illustrated in figure 2 and resemble those described and illustrated by Edward,[4b] McNaught,[6c] King,[6d] Hoyt [7d] and others. The last, among others, pointed out that these changes are nonspecific.

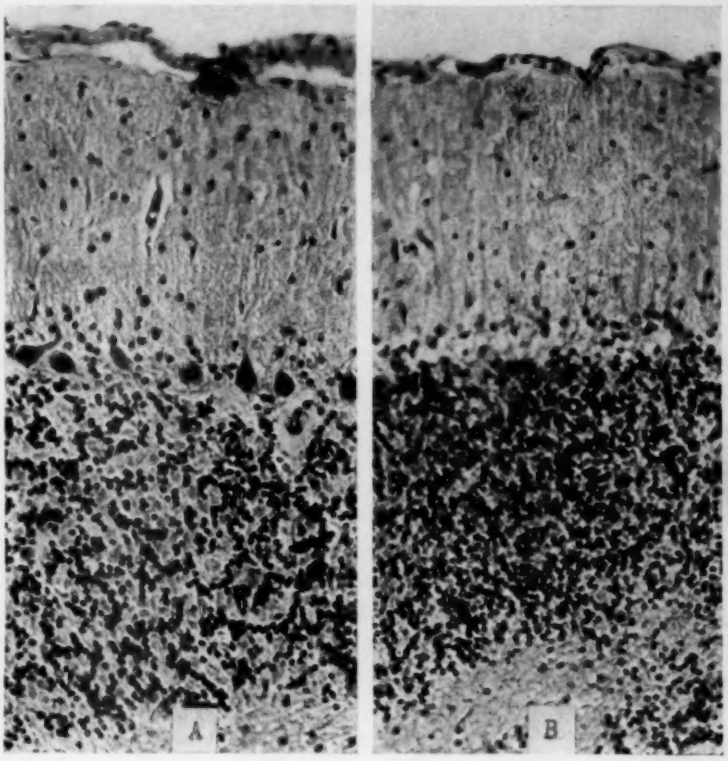

Fig. 2.—*A*, cerebellum of rabbit with a negative Gordon reaction; *B*, cerebellum of rabbit with a positive Gordon reaction, showing complete disappearance of Purkinje cells; × 160.

There is a curious species susceptibility to the agent. Rabbits and guinea pigs are susceptible, while mice,[8] monkeys,[9] kittens,[5d] pups and ferrets [5d] are resistant. The Purkinje cells in the species that fail to give the reaction have not been studied, to my knowledge.

Attempts have been made to correlate the presence of the active agent in diseased lymph nodes with one or more cell types. Gordon

8. Gordon (footnotes 1 *b* and 3 *a*).
9. Gordon.[3a] Smith.[5i]

noticed that very fibrotic nodes gave negative reactions [1b] and that the agent was not associated with any particular cell.[3a] Van Rooyen at first agreed with this observation [5d] but later thought that the agent was related to the presence of reticulum cells.[10] Chapman [5n] and Hoyt [7d] saw no correlation whatever with the histologic character. After careful differential cell counts, Turner, Jackson and Parker [6b] related the active agent to the presence of eosinophils. This was quickly confirmed by Edward,[4b] d'Ovidio and Martín Vucetich,[11] Liebegott,[12] McNaught,[6c] Robb-Smith [7c] and King.[6d] Van Rooyen [13] and Barbieri [14] saw no correlation with the presence of eosinophils. Other myeloid cells are also associated with the agent, as pointed out by Edward [4b] and King.[6d] In my experience some lymph nodes rich in eosinophils have given negative results in rabbit tests, while others having very few eosinophils have been active. Spleens containing Hodgkin's lymphogranuloma with eosinophils have also failed to give positive results in rabbits in 2 cases. Further evidence that cells other than eosinophils may contain the agent is furnished by studies on injection of blood and other tissues.

Biologic materials other than lymph nodes have been tested in the brains of rabbits by the Gordon technic. Human leukocytes,[4a] leukocytic cream from eosinophilic blood,[15] lymphocytes,[4b] myelocytic leukemia blood cream [4b] and leukocyte pus [4b] have been reported as showing the presence of the agent. It is difficult to evaluate some of these reports, because details of technic are often omitted. It is usually not stated what anticoagulant was used while the leukocytes were being harvested. A very small amount of potassium oxalate introduced into the rabbit's brain produces a delayed reaction resembling the Gordon reaction, provided the concentration is optimum. Dog leukocytes are said not to contain the agent,[4a] and human myeloblastic leukemia blood cream is negative for the agent.[4b]

Bone marrow is reported as containing the Gordon agent, and this has been my experience also. Normal bone marrow [16] and the marrow in acute leukemia [17] and pernicious anemia [16] are said to harbor the agent, while it is said to be absent in the bone marrow of rabbits,[18] guinea pigs,[18] cats [16] and horses,[16] but present in chicken marrow.[4b]

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10. van Rooyen, C. F.: Edinburgh M. J. **44**:455, 1937.
11. d'Ovidio, F. R., and Vucetich, M.: Rev. Asoc. méd. argent. **52**:1053, 1938.
12. Liebegott, G.: (*a*) Verhandl. d. deutsch. path. Gesellsch. **31**:459, 1938; (*b*) Ztschr. f. d. ges. exper. Med. **105**:241, 1939.
13. van Rooyen (footnotes 5 *d* and 10).
14. Barbieri, D.: Klin. Wchnschr. **18**:1394, 1939.
15. Turner and others.[6b] Edward.[4b] McNaught.[6c]
16. Friedemann, U., and Elkeles, A.: Brit. M. J. **2**:1110, 1933.
17. van Rooyen.[5d] Friedemann and Elkeles.[16]
18. Edward.[4b] Friedemann and Elkeles.[16]

Normal human spleen is said to contain the agent,[4a] as does the mouse spleen,[4b] while rat [4b] and dog [4a] spleens give no reaction. Normal human kidney is stated to give negative results,[4a] while the renal cortex in subacute glomerulonephritis with eosinophils gives positive results.[6c]

The list of materials which give positive results in rabbit tests can be augmented if those reports are included in which the preparations were made by the Jochmann and Lockemann method.[19]

From this incomplete list of bloods and tissues which have been used it is evident that the agent is present in some normal and some pathologic tissues of several species of animals which do not have Hodgkin's disease and that it is difficult to correlate this distribution with any one type of cell. This widespread distribution makes it unlikely that the agent is the causative factor for Hodgkin's disease. The presence of the agent in this wide assortment of tissues does not, however, diminish the value of the reaction as a practical diagnostic test in the case of human lymph nodes, although it does indicate that the reaction is a nonspecific reaction.

Three views have arisen with respect to the nature of the Gordon agent. It has been considered to be a virus, a neurotoxin and an enzyme. Gordon expressed the opinion that the agent is a virus, and he published photographs illustrating elementary bodies.[3a] He further expressed the opinion that the increase in activity during the eight day period of incubation might indicate multiplication of a virus. Against the virus nature have been the failure of serial transmission from one rabbit to another, the absence of immunity in rabbits after recovery, the absence of complement-fixing antibodies or precipitins in the rabbit serum,[7a] the absence of neutralizing factors in the patient's serum against the agent [7a] and the failure of a supercentrifuge to sediment the agent.[4c]

Van Rooyen [5c] suggested that the agent might be a specific neurotoxin. Although it might be a toxin, its known distribution casts doubt on its being specific.

Friedemann [4a] pointed out that in its distribution and properties the Gordon agent resembles Jochmann's proteolytic ferment. MacKenzie and van Rooyen [20] and Wurm [5c] stated that these agents were not identical. Kelser and King,[19] working with marrow extracts by the Jochmann method, held that the agents were identical without having worked with the Gordon agent.

Other properties of the Gordon agent which might give clues as to its nature have been mentioned by Gordon,[1] van Rooyen,[21] MacKenzie and van Rooyen,[20] McNaught [6c] and others. Its ability to withstand adverse conditions makes it highly improbable that the agent is living.

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19. Kelser, R. A., and King, L. S.: Am. J. Path. **12**:317, 1936.
20. MacKenzie, I., and van Rooyen, C. E.: Brit. M. J. **1**:406, 1935.
21. van Rooyen (footnotes 5 *a*, *c* and *d*).

SUMMARY

The Gordon reaction was positive in 16 (76.2 per cent) of 21 cases of Hodgkin's lymphogranuloma. Of 310 cases previously reported in the literature, the test was positive in 229 (73.9 per cent). In my experience with 40 control (nonlymphogranulomatous) lymph nodes there were no positive reactions, while of 452 control cases collected from the literature, positive reactions were reported in 8 (1.77 per cent). A critical review of these 8 cases reveals that in most, if not all, of them the involved tissue could have been differentiated from Hodgkin's lymphogranuloma by histologic criteria. The Gordon reaction is consequently reliable in differential diagnosis of lymph node diseases if it is accompanied by histologic examination, and the test shows uncanny ability in differentiating closely related types of lymphadenopathy.

The distribution of the Gordon agent is such as to make it unlikely that it is the causative agent of the disease. Its properties are those of a nonliving agent, probably enzymatic. The test, while reliable, is therefore nonspecific.

EXPERIMENTAL STUDIES IN CARDIO-VASCULAR PATHOLOGY

III. POLYVINYL ALCOHOL ATHEROMATOSIS IN THE ARTERIES OF DOGS

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The experimental production of atheromatosis in animals has been limited so far to the use of one method, i. e., oral administration to rabbits and possibly to guinea pigs of a diet rich in cholesterol. All attempts to elicit such vascular responses by this means in other animals, especially dogs and cats, have been unsuccessful.[1] These failures are attributed in general to the fact that dogs and cats excrete any excess of ingested cholesterol relatively rapidly, and therefore alimentary hypercholesteremia does not develop, while rabbits are apparently incapable of eliminating this material quickly and hence it accumulates in the blood and other tissues, creating thereby a condition which favors cholesterol infiltration of the vascular wall.

In an investigation of the effects on the blood of repeated intravenous or intraperitoneal injections of polyvinyl alcohol[2] it was observed on examination of the organs of the dogs used in the experiments that atheromatous lesions could be produced in the arteries by the introduction of a chemical which behaves in the body in many respects like cholesterol though not occurring naturally in the animal organism. These observations are reported not only because they open a new, rapid method for the experimental production of atheromatosis in animals but also because they furnish important evidence concerning the formal and the causal genesis of atheromatosis and arteriosclerosis.

EXPERIMENTS

Eight adult dogs were used in the experiments. Four received injections in the jugular vein of a 5 per cent polyvinyl alcohol solution in physiologic solution of

From the Warner Institute for Therapeutic Research.

1. (*a*) Anitschkow, N.: Experimental Arteriosclerosis in Animals, in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 271. (*b*) Duff, G.: Am. J. Path. **8**:219, 1932; Arch. Path. **20**:81 and 259, 1935; **22**: 161, 1936. (*c*) Leary, T.: ibid. **17**:453, 1934.

2. Hueper, W. C.; Landsberg, J. W., and Eskridge, L.: J. Pharmacol. & Exper. Therap. **70**:201, 1940. Martin, G., and Hueper, W. C.: J. Biol. Chem., to be published.

sodium chloride, while the other 4 were given the polyvinyl alcohol solution intraperitoneally.

Intravenous Series.—Dog 209, weighing 11.1 Kg., received daily doses—on the first day 15 cc. of the polyvinyl alcohol solution, on the second and third day 65 cc. and on each of the following two days 70 cc., a total of 285 cc. within five days. From the beginning of the experiment the dog showed loss of appetite and rapid decrease in weight, caused in part by a bloody diarrhea accompanied by frequent vomiting, which developed after the first injection. On the sixth day of the experiment the dog was moribund and was killed.

Dog 217, weighing 10.3 Kg., received a daily dose of 70 cc. of the polyvinyl alcohol solution from the first to the fourth day, inclusive, from the seventh to the tenth day and on the fourteenth day of the experiment (total, 630 cc.) and was then killed with ether.

Dog 212, weighing 10.3 Kg., was given a total of 1,145 cc. of polyvinyl alcohol solution, injected in daily amounts of 65 to 70 cc. from the first to the fourth, the seventh to the tenth, the thirteenth to the seventeenth and the twentieth to the twenty-third day of the experiment. Death occurred on the twenty-fourth day. During the time of treatment the dog became anoretic and mentally dull.

Dog 2, weighing 9.0 Kg., was given a total of 915 cc. of polyvinyl alcohol solution, 20 cc. of which was injected on the first day, 20 cc. on the second day, 35 cc. on the third day and 60 cc. on the fourth day and daily from the seventh to the eleventh, from the fourteenth to the eighteenth and from the twenty-first to the twenty-third day. The dog died on the twenty-fourth day.

Intraperitoneal Series.—Dog 218, weighing 9.7 Kg., was given 70 cc. of polyvinyl alcohol solution on the first, second, sixth, eighth, tenth, thirteenth and fifteenth days of the experiment, receiving thus a total of 490 cc. of a 5 per cent polyvinyl alcohol solution. The dog was killed on the ninety-second day.

Dog 219, weighing 10.0 Kg., was given the same dose and at the same intervals until a total dose of 490 cc. of the solution had been administered. The dog was killed on the twenty-third day of the experiment.

Dog 215, weighing 11.8 Kg., received 60 cc. of the solution on the first, fourth, seventh, ninth, eleventh, fourteenth and sixteenth days, 65 cc. on the eighteenth day and 70 cc. on the twenty-first, twenty-third and twenty-eighth days, (total, 695 cc.) and was killed on the thirty-third day.

Dog 267, weighing 14.5 Kg., was given on the first day 300 cc. of a 5 per cent polyvinyl alcohol solution, on the third day, 250 cc., on the eighth day 375 cc., on the tenth day 300 cc. and on the thirteenth, fifteenth, seventeenth and twenty-first days 375 cc. (total, 2,725 cc.). The dog died on the twenty-first day of the experiment.

It was noted that in the dogs of both series there was a tendency for ill defined soft subcutaneous nodules to develop, especially at the sites of physiologic trauma, resembling in appearance and localization the xanthomatous nodes observed in rabbits following an oral feeding with cholesterol. There appeared, moreover, a marked hemorrhagic diathesis which was expressed in hemorrhages from the skin and mucous membranes on slight provocation and which was associated with a prolonged bleeding time. The hematologic studies revealed the presence of more or less marked anemia in the treated dogs, characterized by a reduction in the number of erythrocytes and a proportionally even more pronounced decrease in

the amount of hemoglobin. The sedimentation and conglomeration of the erythrocytes was much accelerated, while the clotting of the blood was much delayed or at times even completely suppressed. The viscosity of the whole blood as well as of the plasma was considerably elevated. The polyvinyl alcohol was demonstrable in blood smears stained with compound solution of iodine U. S. P. (Lugol's solution) (Hueper [3]) and in some instances was present in such amounts that it appeared as if up to 75 per cent of the liquid parts of the blood consisted of polyvinyl alcohol. This chemical was retained in the circulating blood to a considerable degree over periods up to several months. The number of platelets was reduced in many instances. During the late stages of polyvinylization the urine contained appreciable quantities of albumin. There were no significant changes in the prothrombin, cholesterol and calcium levels of the blood.

Pathology.—The meningeal membranes and the brain were normal in appearance, size and consistency. The lungs were often edematous and congested. The heart showed a contracted left ventricle. The aorta was smooth. The peritoneum was smooth and glistening. An appreciable amount of clear, watery ascitic fluid was present in dogs 215 and 267, which had received intraperitoneal injections of polyvinyl alcohol solution and which showed also general subcutaneous edema, moderate bilateral hydrothorax and hydropericardium. The liver was firm and brown and in several dogs contained a delicate grayish network. The livers of dogs 209 and 212 were moderately enlarged (389 Gm. and 432 Gm., respectively). Both these dogs exhibited also marked increase in the size of the spleens (285 Gm. and 113 Gm., respectively, compared with 79 Gm. in dog 2 and 40 to 45 Gm. in the other dogs). The spleen of dog 206 was dark red and firm, while that of dog 212 was pinkish gray and soft, the pulp being almost liquid. The adrenals were normal in size and appearance. The kidneys of dogs 209, 212 and 218 were small, greenish to brownish slate gray and coarse granular on the cortical surface. Edematous, congested or hemorrhagic areas were encountered in the mucosa of the small intestines of dogs 209, 212 and 217. The mesenteric and retroperitoneal lymph nodes were normal or moderately enlarged and grayish pink or brown. Testes, prostate, bladder and bone marrow were normal, while jelly-like and often hemorrhagic foci were found not infrequently in various parts of the subcutaneous tissue.

Histology.—The histologic changes observed in the various organs were in many respects identical or similar in type, but not always in degree, to those reported previously in connection with a study of the effects of subcutaneous injections of a solution of polyvinyl alcohol in rats.[3] They may be summarized, therefore, for most of the organs.

Hypophysis: This gland was normal.

Meningeal Membranes and Brain: Globules of polyvinyl alcohol were demonstrated in the lumens of some meningeal and cerebral vessels. Fine droplets of this substance were found in histiocytic foam cells of the choroid plexus. In none of the brains was polyvinyl alcohol found within the nervous substance. There were, however, perivascular zones of debris, hemorrhages, areas of gliosis and foci of vacuolar ganglion cell degeneration and of ameboid glia.

Thyroid and Parathyroid: The interfollicular capillaries of the thyroid were filled extensively with globules and casts of polyvinyl alcohol. Fine droplets of

3. Hueper, W. C.: Arch. Path. **28**:510, 1939.

polyvinyl alcohol were visible within the colloidal content of the moderately sized follicles. A dustlike accumulation of polyvinyl alcohol was present in small groups of foam cells in the interstitial tissue.

Lungs: Multinucleated giant cells, multinucleated syncytia and foamy proliferated endothelial cells lined the lumens of some smaller pulmonary vessels. Films and granules of polyvinyl alcohol covered the inside of many vascular lumens, while precapillary and capillary vessels contained not infrequently solid polyvinyl alcohol casts. Granules and films of polyvinyl alcohol coated in part the walls of the alveoli. Droplets of this material were found within the serous exudate filling the alveoli in places or were mingled with the cellular content of bronchiolar and bronchial lumens.

Heart: The myocardium was normal in 6 dogs, whereas minor degenerative changes of the muscular elements as well as interstitial edema were observed in the remaining 2 dogs. Small foci of foam cells containing polyvinyl alcohol globules were seen occasionally in the interstitial tissue.

Liver: The liver cell cords were narrow and composed of atrophic hepatic cells, pushed apart by proliferated and markedly swollen Kupffer cells that frequently formed small granulomatous nodes consisting of multinucleated giant cells and foam cells. Necroses of liver cells were seen in some dogs. The liver of dog 267 was completely necrotic. Large amounts of polyvinyl alcohol were present in the balloon-like swollen Kupffer cells. Foam cells with droplets of polyvinyl alcohol were visible in the periportal connective tissue.

Pancreas: Scanty blue-colored droplets of polyvinyl alcohol were found in interstitial foam cells and in the vascular lumens.

Stomach: Globules of polyvinyl alcohol were visible in the vascular lumens of the mucosa.

Intestine: Small mucosal hemorrhages and leukocytic infiltrations were found in dog 209. Sections (stained with compound solution of iodine) of the intestines of all dogs showed, however, that there was a massive intracellular and extracellular infiltration of the intestinal wall by polyvinyl alcohol involving mainly the submucosa and the basal portions of the mucosa, where polyvinyl alcohol seemed to be present within the mucous glands.

Spleen: The pulp consisted of a more or less dense accumulation of foam cells mixed with multinucleated giant cells and syncytia. Some of these cells possessed nuclei of enormous size. The pulp of the spleens of dogs 209 and 267 was completely necrotic and showed hemorrhagic imbibition. The follicles were highly atrophic. Golden brown extracellular and intracellular pigment was found within the pulp in several cases.

Lymph Nodes: Numerous foam cells containing droplets of polyvinyl alcohol were observed in the distended sinuses. In some nodes there had been a complete transformation of the lymphoid tissue into a foam cell tissue with numerous multinucleated giant cells present.

Marrow: In most dogs the marrow of the sternum and femur was of a dense immature myeloid type, showing globules of polyvinyl alcohol within the reticulum cells. The marrow of dogs 2 and 267, however, consisted of a loose fat marrow containing small accumulations of lymphoid cells.

Adrenal Glands: There were hyperplasia and swelling of the reticulum cells present, which were filled with large amounts of polyvinyl alcohol. The adrenals of dog 209 were destroyed by bilateral extensive hemorrhages into their substance.

Kidneys: The endothelial cells of the glomeruli were in many instances balloon-like, swollen and transformed into vesicles filled with a fine granular matter. The capillary lumens were obliterated thereby, causing atrophy, fibrosis and hyalinization of large multicystic glomeruli resembling bunches of unequally sized grapes. Some of these glomeruli were adherent to thickened Bowman's capsules. The tubular epithelium was more or less markedly degenerated, while the lumens were filled with cellular debris and a homogeneous, deep pink-stained material, sometimes containing blue, calcified granules. The interstitial tissue was broadened and infiltrated with a homogeneous material of mucoid appearance. Foamy histiocytes and mononuclear cells were found in wedge-shaped areas of fibrosis and atrophy, producing the morphologic picture of chronic nephrocirrhosis. Polyvinyl alcohol was demonstrable in the various lesions, described in varying amounts, being located intracellularly as well as extracellularly.

Bladder: The bladder was normal in all dogs except one, 209, in which the subserous tissue contained a dense capillary network with hemorrhages in which much polyvinyl alcohol was found, while multinucleated giant cells lined the lumens of some of the submucosal vessels.

Prostate: This gland was normal in some dogs; in others it showed cystic hyperplasia.

Testes: Marked diffuse or localized atrophy of the spermatogenic epithelium and a varying number of multinucleated spermatic giant cells were observed. There were areas of complete desquamation of the lining cells of the spermatogenic tubules, associated with a partial collapse of the empty tubules. Polyvinyl alcohol was demonstrated within some of the testicular vessels.

Epididymis: This was normal with completely or partially empty lumens.

Subcutaneous Nodes: An inflammatory granulation tissue was composed of fat cells, foam cells, multinucleated giant cells and focal necroses and hemorrhages. Polyvinyl alcohol was demonstrable in fat cells and in the various histiocytic elements.

Vascular Lesions: Apart from the vascular changes of a foreign body type of reaction which were seen in the vessels of the lung and bladder, there occurred alterations which were of a character usually considered indicative of atheromatosis. These lesions involved the large as well as the smaller vessels of the body (aorta, femoral artery, carotid artery, coronary vessels, renal vessels), i. e., arteries of the elastic as well as of the muscular variety.

1. Sections stained with hematoxylin-eosin—(*a*) elastic vessels (aorta and carotid and femoral arteries): Sections prepared from the ascending and thoracic parts of the aorta and from the carotid and femoral arteries showed in many instances a balloon-like swelling of the endothelial cells which lined the inner wall either in a single layer or in several layers and which had the character of foam cells. The media was often unchanged during this stage but in several cases showed in the region of the inner third a loosening of the structure brought about by an infiltration of a homogeneous, unstained material in between the muscle cell bundles (fig. 1 *A* and *B*). In somewhat more advanced lesions there was a narrow accumulation of foamy histiocytes between the endothelium and the media (fig. 1*C*). Still older lesions exhibited a marked cushion-like thickening of the intima consisting of foam cells and occasionally containing a small capillary vessel (fig. 2 *A*). It was not unusual that during this stage smaller or larger masses of foam cell–like histiocytes were present in the inner third of the media, causing marked loosening and distortion of the pattern of the aortic wall. The topographic relation of

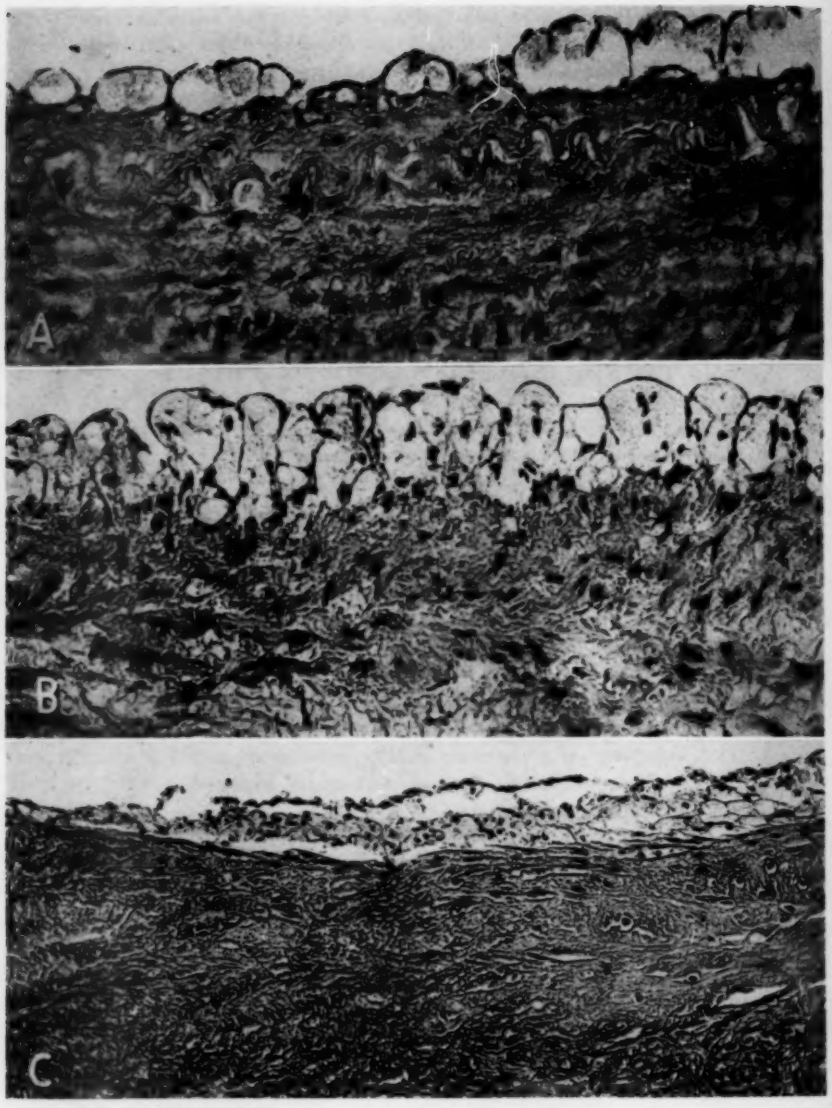

Fig. 1.—*A*, aorta lined with balloon-like distended foamy endothelium, containing droplets of polyvinyl alcohol. *B*, aorta lined with several layers of these swollen endothelial foam cells. The inner part of the media contains polyvinyl alcohol in the interstices of the muscle bundles. *C*, aorta with a narrow cushion-like thickening of the intima, consisting of foamy endothelial histiocytes.

Fig. 2.—*A*, aorta just above the aortic valve with a thick foam-cellular cushion of the intima containing a small capillary vessel. *B*, small polypous foam-cellular intimal thickening of a femoral artery with beginning polyvinyl alcohol infiltration and foam cell invasion of the media.

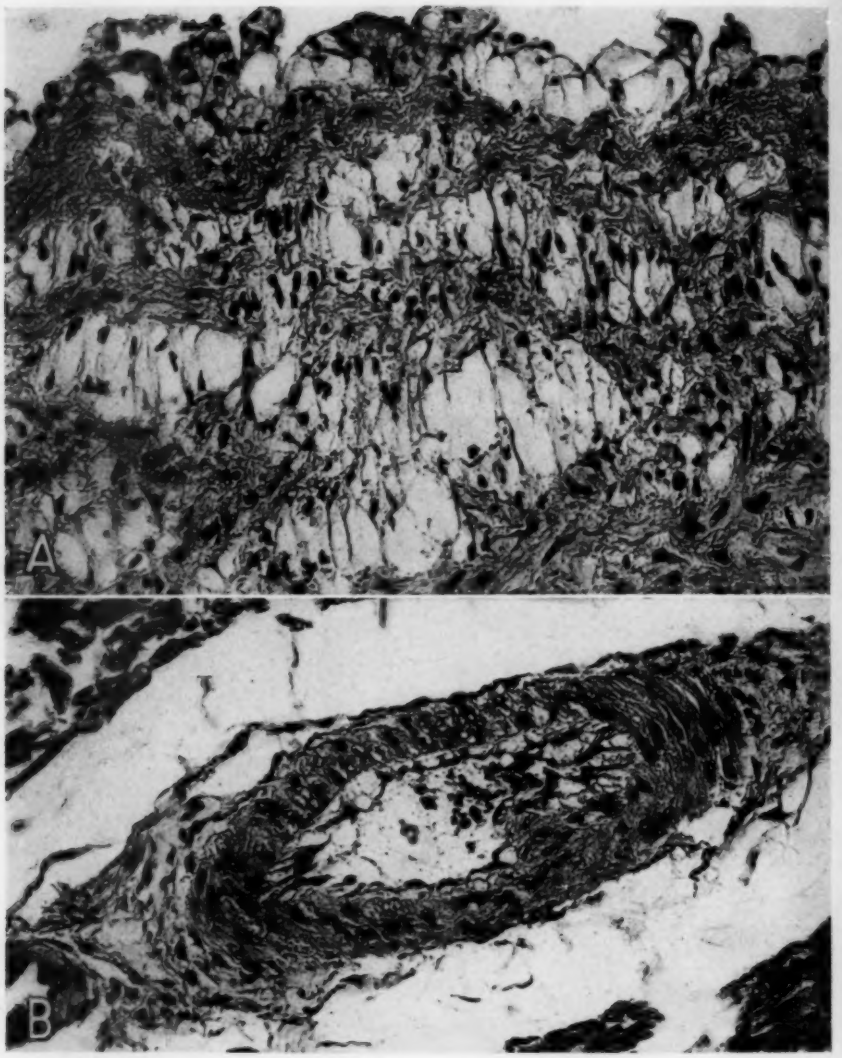

Fig. 3.—*A*, aorta with stratified foam-cellular intima and diffuse foam cell invasion of the media, resulting in marked distortion of the arrangement of the muscle bundles. *B*, coronary artery with small foamy endothelial cells and vacuolated media.

these foam cells to the endothelial lining, from which they seemed to extend, made it probable that these histiocytic cells were derived from the endothelial cells (figs. 2 *B* and 3 *A*). Many of these lesions were found in the region just above the aortic valves, as these parts of the aorta were routinely included in the sections of the left ventricular wall of the heart.

(*b*) Coronary Vessels: The walls of the coronary arteries were sometimes swollen and edematous, exhibiting marked vacuolation of the media, which contained groups of mononuclear cells and areas of hyalinization. They occasionally showed small areas of swollen endothelial foam cells (figs 3 *B* and 4).

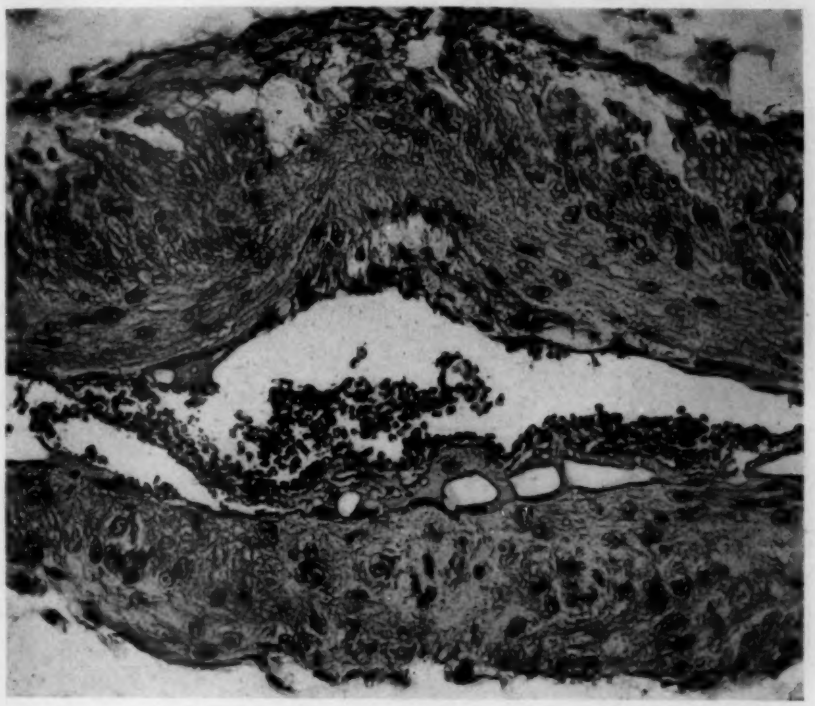

Fig. 4.—Coronary artery with a small focus of endothelial foam cells, swelling of the media and infiltration by histiocytic cells.

(*c*) Renal Vessels: Similar changes were observed in the renal arteries. The main branches of the renal artery, situated in the region of the hilus sometimes displayed larger foci of hyalinization in the media, while the smaller renal arteries and arterioles in the parenchyma exhibited relatively regularly marked vacuolation of the media, resulting from vesiculation of swollen muscle cells (fig. 5 *A*). There was, moreover, not infrequently, hyaline thickening of the arteriolar walls, accompanied by a foam-cellular transformation of the endothelium (fig. 5 *B*).

2. Sections stained with compound solution of iodine and sudan III: The sections of the various large arteries and those of the heart and kidney were stained with compound sclution of iodine and with sudan III for the determination of the

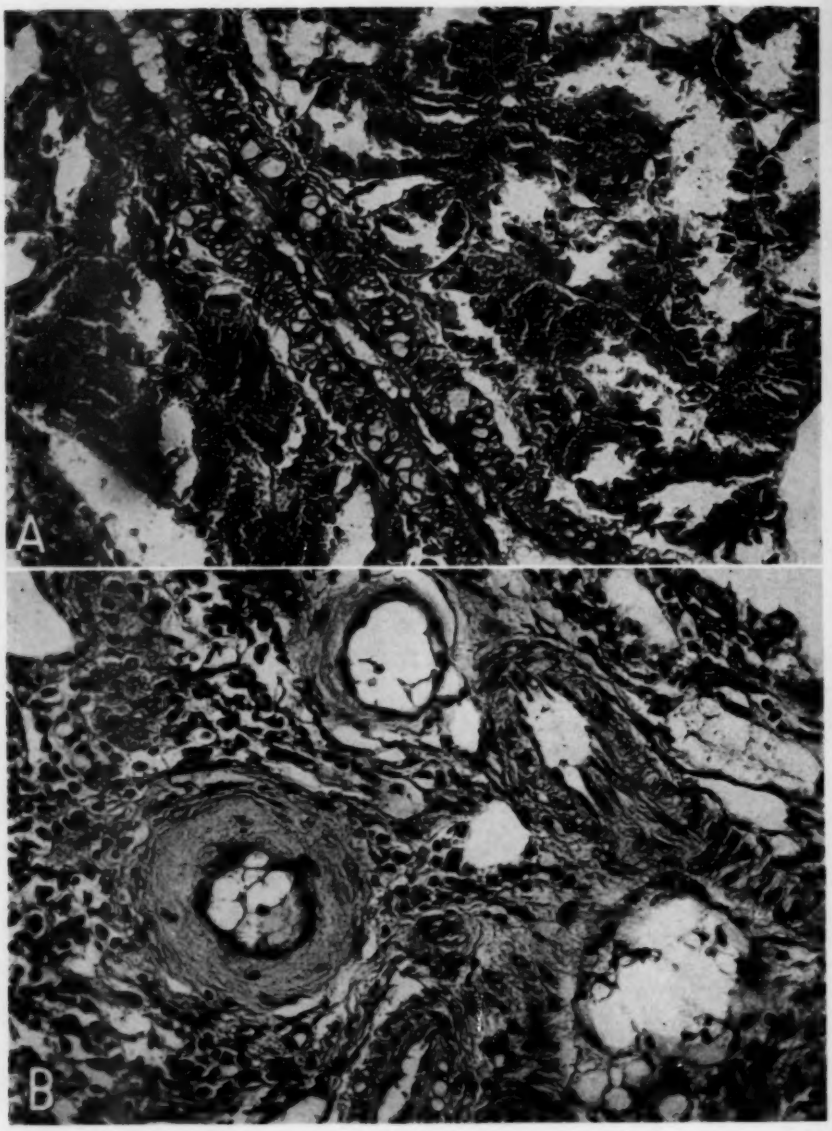

Fig. 5.—*A*, renal artery with marked vacuolation of the media. The vacuoles in the swollen smooth muscle cells contain droplets of polyvinyl alcohol. *B*, renal arterioles with endothelial foam cells and swollen and hyalinized media.

chemical character of the material contained in the endothelial and histiocytic foam cells as well as in the muscle cells and in the interstitial spaces of the media of the arterial walls. The sections stained with compound solution of iodine for the demonstration of polyvinyl alcohol showed the presence of blue globules and granules in varying amounts in the endothelial foam cells as well as in the foam cell-like histiocytes present in the cushion-like thickenings of the intima and as more or less diffuse or focal cellular infiltrations of the inner third of the media of the larger vessels. The media of the elastic arteries exhibited extracellularly located polyvinyl alcohol infiltrating the interstices of the subendothelial portions of the media. Globules of polyvinyl alcohol were demonstrated as the content of the vacuoles of the muscle cells composing the media of the coronary and renal artericles.

Sections stained with sudan III, on the other hand, revealed that the balloon-like swollen endothelial cells and the histiocytic foam cells were free from lipids in most instances. It was observed occasionally that the endothelial foam cells contained a few reddish-stained granules or seemed to be coated toward the lumen of the aorta with a reddish, apparently intracellularly situated film. Reddish globules and granules were found in the media within the interstitial spaces occupied by polyvinyl alcohol as well as underneath the cushion-like intimal thickenings within the muscle cells.

COMMENT

The observations reported show that repeated intravenous or intraperitoneal introduction of an aqueous solution of polyvinyl alcohol into dogs results in the development of arterial lesions closely similar to the atheromatous changes occurring spontaneously in the arteries of man and elicited experimentally in those of rabbits by feeding them a diet rich in cholesterol. The staining of vascular sections with compound solution of iodine and sudan III, respectively, establishes the fact that the material stored or retained in the endothelial and histiocytic foam cells and infiltrating the media is polyvinyl alcohol and is not cholesterol. It appears to be appropriate, therefore, to name the vascular lesion produced "polyvinyl alcohol atheroma" in contrast to the usual cholesterol atheroma. The distribution of the polyvinyl alcohol atheromatosis as well as the variations in type of the vascular changes, depending on the elastic or muscular character of the vessels affected, shows a resemblance to the corresponding vascular alterations occurring in rabbits.

The formal genesis of these conditions seems to be as follows: The large amounts of polyvinyl alcohol introduced are retained to an appreciable degree in the circulating blood, where the alcohol has a tendency, accentuated by the increased plasmatic viscosity, to cover the inside of the vascular walls with a film that is in direct contact with the endothelium. The endothelial cells take up the foreign matter and are transformed thereby into foam cells. Following a reactive proliferation of the damaged endothelial cells and a penetration of the polyvinyl alcohol from the blood through the endothelial lining into the media, foam-cellular endothelial histiocytes invade the inner parts of the media. The reactive endothelial proliferation in turn results in the formation of

cushion-like intimal thickenings, which may be invaded secondarily by capillary vessels. The accumulation of the polyvinyl alcohol in the endothelial histiocytes and in the interstices of the media is followed occasionally by the appearance of smaller amounts of fatty granules within the endothelial foam cells as well as in the muscle cells of the media and in the polyvinyl alcohol infiltrating these parts of the vascular wall. The absence of foam cells around the vasa vasorum and the limitation of the infiltrative processes to the inner third of the media, decreasing in severity toward the adventitia, are considered as conclusive proof that the vasa vasorum or any abnormal intravascular capillary proliferations or any of their contents are not involved in the production of polyvinyl alcohol atheroma.

While these findings are of distinct importance from a pathologic standpoint, the information which they yield in regard to the causal genesis of atheromatosis and arteriosclerosis is of even greater significance. In an evaluation of the evidence in this respect the following points are especially noteworthy: There exists a polyvinylization of the blood of considerable degree and persistence, because the organism does not possess any rapid and effective means of decomposing or eliminating this macromolecular, chemically rather inert compound; polyvinyl alcohol with plasma forms a rather unstable emulsion influencing the physicochemical status of the blood; polyvinyl alcohol coats the intima of vessels with a film; it is taken up readily by a great variety of phagocytic as well as of nonphagocytic cells (reticuloendothelial cells, histiocytes, fat cells, endothelial cells, fibroblasts, muscle cells, tubular epithelium, intestinal epithelium, glia cells, ganglion cell and others), converting these cells into foam cells. It is known from the industrial application of polyvinyl alcohol that films of this substance are relatively impermeable to gases and impervious to oils, greases and fats and are used, therefore, for protective purposes.

These data permit the conclusion that polyvinyl alcohol present in the blood and coating the vascular endothelial lining of blood vessels interferes with a normal and adequate exchange of the various gaseous and liquid nutritive substances and waste metabolites between the blood and the inner third of the vascular walls. The resulting cellular injury is intensified when the polyvinyl alcohol is taken up by the endothelial cells and infiltrates the vascular wall. The reactive proliferation of the endothelial cells and their foam-cellular transformation produces atheromatous lesions. The physicochemical qualities of polyvinyl alcohol films toward fatty substances and the absence of any appreciable amounts of fat-stainable material in the lesions as well as of any hypercholesteremia exclude any significant role of lipids in the development of these vascular changes. The minor fatty infiltrations observed occasionally in the media are obviously of secondary nature. A similar causative mechanism

is evidently active in the production of the polyvinyl alcohol vacuolation and subsequent hyalinization of the media of the coronary and renal vessels.

In comparing human and experimental cholesterol atheromatosis with polyvinyl alcohol atheromatosis it becomes apparent not only that there exist relatively close morphologic similarities between these two types of lesions but that even the causative mechanisms of the two conditions have many points in common.

Cholesterol, similar to polyvinyl alcohol, is a relatively stable substance which is highly resistant to enzymatic degradation and impermeable to many agents. It therefore exerts a certain protective action on cells (Starling;[4] Leary[1c]). Cholesterol resists infection, as evidenced by the absence of such complications in atheromatosis, apparently to the same degree as polyvinyl alcohol.

While present in the plasma, usually in a dissolved form, cholesterol, fatty acid esters of cholesterol and other lipids form a milky emulsion with the plasma when occurring in excess (hyperlipemia), thereby providing an additional resemblance with the physicochemical status of polyvinylized blood. Hyperlipemia, which is observed as a transitory phenomenon following the ingestion of a diet rich in lipids, after hemorrhages and during starvation, represents a more persistent status of the blood in diabetes mellitus, hypothyroidism (myxedema), nephrosis and the chronic active stage of chronic glomerulonephritis.

As the routes of excretion of cholesterol are restricted normally, this material is accumulated in the various organs during prolonged periods of pathologic hypercholesteremia, and it is under such conditions that an increased and precocious development of atheromatosis occurs in man and animals (diabetes mellitus, myxedema, chronic glomerulonephritis, nutritive hypercholesteremia). The occurrence of these vascular degenerative lesions in hypothyroidism of man and animals (Falta[5]) and in diabetes mellitus appears to be particularly significant concerning their causal genesis, as both diseases are characterized by impairment of the oxidative metabolism which is apt to aggravate the nutritive disturbances set up in the vascular walls by the lipemic condition of the blood. The connection of the causation of atheromatosis with impairment of the oxygenative metabolism is further emphasized by the fact that it is possible to improve appreciably the lipemic condition of myxedematous persons by the administration of thyroxin U. S. P. in amounts influencing markedly the oxidative metabolism and that the

4. Starling, E. H.: Principles of Physiology, Philadelphia, Lea & Febiger, 1930, p. 42.

5. Falta, W.: Die Erkrankungen der Blutdrüsen, in Mohr, L., and Staehelin, R.: Handbuch der inneren Medizin, ed. 2, Berlin, Julius Springer, 1927, vol. 4, pt. 2, p. 1075.

development of atheromatosis in rabbits by cholesterol feeding can be prevented by similar medication (thyroxin or iodine) through its action on the metabolism (Anitschkow [1a]).

These observations and considerations indicate that cholesterol atheromatosis and polyvinyl alcohol atheromatosis have approximately the same causative mechanism, represented fundamentally by a disturbance of the nutritive and oxidative metabolism of the vascular wall, such as was demonstrated recently in connection with the experimental production of hypotensive arteriosclerosis (Hueper and Landsberg [6]) and that atheromatosis is merely a variety of arteriosclerosis which receives its special morphologic features from the presence and accumulation of a chemically rather inert causative substance.

SUMMARY

In dogs repeated intravenous and intraperitoneal injections of a 5 per cent aqueous solution of polyvinyl alcohol result in the development of atheromatous lesions in the aorta and in the carotid and femoral arteries as well as in the appearance of this chemical within the media of smaller arteries and arterioles of the heart and kidney.

The polyvinyl alcohol atheromatosis is morphologically similar to the cholesterol atheromatosis observed in man and rabbits.

The causative mechanism of both types of atheromatosis is represented by a disturbance of the nutritive and oxidative metabolism of the vascular walls brought about by the physicochemical changes prevailing in polyvinylized blood as well as in hyperlipemic conditions.

6. Hueper, W. C., and Landsberg, J. W.: Arch. Path. **29**:633, 1940.

FALSE DIVERTICULUM FORMATION FOLLOWING ACUTE PERFORATIVE APPENDICITIS

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From a study of 85 false diverticula in 49 appendixes, it appears that false diverticulum formation follows acute perforative inflammation, usually along the mesoappendical border and usually in the distal third of the organ. The inflammatory process extends through the appendical wall and causes necrosis, liquefaction and cavitation of the regional adipose tissue. When the inflammatory process subsides, there is a growth into the cavity in the fat and subsequent development there of mucosa, submucosa and subserosa derived from the coats regional to the area of perforation. Although much smooth muscle in the originally inflamed area of the appendix is destroyed, there is no appreciable regeneration of it and no extension of it into the wall of the false diverticulum. The false diverticulum of the appendix, with no muscle in the wall, is easily distinguished from the very rarely observed true diverticulum, with all coats present. At any stage in its development the false diverticulum may become inflamed and occasionally may perforate. Not infrequently there is gradual atrophy of the wall of the false diverticulum, especially of the mucosa, a process of involution, just as in the appendix itself (fig. 1).

Although the abnormality was described as early as 1842, by von Rokitansky,[1] an accurate and comprehensive description of the developmental, inflammatory and regressive changes of false diverticulum of the appendix is still lacking in the literature.

False diverticula have been reported in about 1 in 500 to 1 in 30 appendixes removed at operation or at autopsy (0.17 to 3.56 per cent).[2] In many of the recorded cases the diverticula were multiple and measured from a few millimeters to about 1 cm. in diameter. Pack and Scharnagel[3] reported a case in which there were 36 false diverticula of the appendix.

From the Department of Laboratories, Mount Sinai Hospital.

1. von Rokitansky, C.: Wien. med. Wchnschr. **16**:863, 1866; cited by Sauer, P. K.: Am. J. Surg. **10**:564, 1930.

2. Collins, D. C.: Ann. Surg. **104**:1001, 1936.

3. Pack, G. T., and Scharnagel, I.: Am. J. Surg. **5**:369, 1928.

Our study is based on the examination of 85 false diverticula in 49 appendixes in a series of about 10,000 appendixes observed at the Mount Sinai Hospital and the Woman's Hospital in the past twelve years (incidence about 0.49 per cent). Most frequently the diverticula were found in the distal third of the appendix. They were observed

Analysis of the Data on Forty-Nine Cases of False Diverticulum of the Appendix (Total Number of Diverticula, Eighty-Five)

Sex (42 cases)		
Male	11 cases	(26.1%)
Female	31 cases	(73.9%)
No data	7 cases	
Age (42 cases)		
Youngest, 13 years; oldest, 68 years		
Distribution: 10 to 19 years	4 cases	
20 to 29 years	11 cases	
30 to 39 years	9 cases	
40 to 49 years	9 cases	
50 to 59 years	5 cases	
60 to 69 years	4 cases	
(Total, 42 cases; no data on 7 cases)		
Number of diverticula per appendix (49 cases)		
26 cases with 1 diverticulum	26	
13 cases with 2 diverticula	26	
8 cases with 3 diverticula	24	
1 case with 4 diverticula	4	
1 case with 5 diverticula	5	
(Total, 49 cases; 85 false diverticula)		
Location of false diverticula		
Distal third	46 diverticula	(79.3%)
Middle third	8 diverticula	(13.8%)
Proximal third	4 diverticula	(6.9%)
Not stated	27 diverticula	
(Total, 85)		
Site of false diverticula		
Mesoappendical	66 diverticula	(83.4%)
Antimesoappendical	4 diverticula	(5.1%)
Sides	9 diverticula	(11.5%)
Not stated	6 diverticula	
(Total, 85)		
State of false diverticula		
Early	8 diverticula	
Moderately advanced	4 diverticula	
Fully developed	15 diverticula	
Involuted	30 diverticula	
Inflamed—moderately advanced	2 diverticula	
Inflamed—fully developed	18 diverticula	
Inflamed—involuted	4 diverticula	
Not stated	4 diverticula	
(Total, 85)		

in various stages of development and regression. Some were acutely inflamed, and in a number there was perforation. The false diverticula were frequently multiple (2 to 5) and in the same or different stages of development, inflammation or regression (table). The diverticula varied from a few millimeters to 1 cm. in the large diameter. Well developed false diverticula showed no trace of the inflammatory exuda-

tion of the original perforative appendicitis. Such diverticula are apparently similar to those which previous observers considered were formed by herniation of mucosa and submucosa through preformed or acquired apertures in the muscle. Some of those reported as true diverticula [4] were quite possibly false diverticula observed long after the original inflammation had completely subsided. Likewise, involuted diverticula were considered previously to represent herniations of submucosa only through apertures or perforations, or as diverticula following inflammation during which the mucosa was completely destroyed (cicatricial diverticulum).[5] Involuted false diverticulum has also been reported as neuroma of the appenedix.[6]

DEVELOPMENTAL, INFLAMMATORY AND REGRESSIVE CHANGES OF FALSE DIVERTICULUM OF THE APPENDIX

The initial change in false diverticulum formation is acute appendicitis, usually focal or segmental, most frequently in the distal third of the organ, with perforation of the walls, usually along the mesoappendical border, but occasionally opposite this site or elsewhere. The probable explanation of the greater frequency of perforation along the mesoappendical border is that there is least resistance to the extension of the acute inflammation of the appendix along the course of the lymphatics and blood vessels in the wall regional to the mesoappendical border and that the spongelike smooth muscle network here, about the large vessels and nerves, is more readily destroyed than are the dense muscular coats elsewhere in the wall.

By the time the perforation occurs along the mesoappendical border, the inflammation has extended into the regional adipose tissue, where, following intense colonization by intestinal bacteria, inflammatory exudation, fat necrosis and liquefaction result. Areas of liquefied fat, degenerated inflammatory exudate and necrotic fixed tissue coalesce. Soon there is well defined cavitation of the inflamed fat, and such areas in places are continuous with the lumen of the appendix by one or more narrow perforations of the inflamed wall. This beginning false diverticulum formation is shown in figures 1 and 1 *B*, a representative case. The adipose tissue shows the changes mentioned. The small areas of cavitation in it contain innumerable bacteria, inflammatory exudate partially or wholly degenerated, remains of necrotic framework of original adipose

4. (*a*) Hedinger, E.: Virchows Arch. f. path. Anat. **178**:25, 1904. (*b*) Mertens, V. E.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **9**:743, 1902. (*c*) Schminke, A.: Arch. f. klin. Med. **254**:771, 1924. (*d*) Sturm, H.: Frankfurt. Ztschr. f. Path. **16**:456, 1915. (*e*) Bachlechner, K.: Zentralbl. f. Chir. **51**:2753, 1924. (*f*) Malone, B.: J. A. M. A. **84**:141, 1925.

5. Letulle, M., and Weinberg, M.: Arch. d. sc. méd. **2**:360, 1897.

6. Dordick, J. R.: Arch. Path. **27**:735, 1939.

tissue, and necrotic and liquefied fat. The irregular perforation of the inflamed wall of the appendix is narrow, and there is no evidence of healing anywhere in the involved area. In addition to the narrow perforation shown, there were numerous others along the mesoappendical border, associated with inflammation, liquefaction and cavitation of the regional fat.

A further stage in false diverticulum formation is shown in figures 2 and 2 *B* (early stage). Here the cavity in the adipose tissue and the perforated area of the appendix are well defined. The wall of the diverticulum opposite the perforation is now composed of inflamed adipose tissue showing beginning fibrous tissue replacement. This fibrous tissue is continuous with similar tissue of the submucosa and subserosa regional to the perforation. At the margin of the perforation the mucous membrane covers the muscle edges and shows beginning ingrowth into the cavity produced by the inflammation of the adipose tissue. The ingrowing epithelial cells, in a single layer or a few layers, extend into the cavity for a small distance, whereas there are none at all as yet in the area opposite the perforation. A small amount of inflammatory exudate is still present in the false diverticulum. Figures 3, 3 *B*, 4 and 4 *B* show still further developmental stages (moderately advanced stage). Here the ingrowth and development of mucosa, submucosa and subserosa are clearly seen. The entire cavity is lined by these newly formed coats. Strikingly apparent is the absence of ingrowth or development of muscle in the wall of the false diverticulum. In these moderately advanced diverticula there are still visible some remains of the original inflammatory exudate.

The fully developed false diverticulum is well shown in figures 5 and 5 *B*. Here the mucosa of the false diverticulum is as well developed as that of the appendix itself. Likewise the submucosa and subserosa are thick. Very little, if any, smooth muscle, however, is present in the wall of the fully developed false diverticulum. No trace of the original inflammatory exudate remains.

In the series there is a case of healed cystlike ulceration (few millimeters in diameter) of the wall, continuous with the lumen and lined by well developed mucosa and submucosa. This may be considered an intramural diverticulum. A similar but larger intramural diverticulum (about 1 cm. in diameter) shows acute inflammation and ulceration of the wall. There are also similar acute inflammation and ulceration of the wall of the regional portion of the appendix.

In the series of 85 false diverticula there are 39 moderately advanced or fully developed ones showing recent inflammation of the wall with regional acute peritonitis (figs. 6 and 6 *B*, a representative case). Some of these diverticula showed perforation of the walls in addition (figs. 7 and 7 *B*). In some the regional portion of the appendix was also inflamed.

In quite a number of the cases of early to well developed false diverticula (inflamed and not inflamed) the lumen of the regional portion of the appendix appears eccentric (figs. 2, 4 and 7). In others it is central (figs. 1, 3, 5 and 6).

Before discussing the regressive changes which false diverticula undergo, it is advisable to discuss similar changes involving the appendix itself.

As pointed out as long ago as 1893 by Ribbert,[7] many human appendixes, after childhood especially, show progressive diminution of lymphoid, epithelial and stromal tissues of the mucosa until no glands and no lymphoid tissue remain in this coat. The lumen becomes progressively smaller in the involved area, and finally is obliterated. There is also some atrophy of the outer coats, and the appendix, originally about 5 mm. thick, is now 3, 2 or even only 1 mm. thick. Ribbert considered the process to be an involution of the organ. In cases in which there is evidence of previous or coincident inflammation or edema, the thickness of the appendix in the involuted area may exceed 5 mm. Involution usually involves the distal third of the appendix (the tip most frequently), but the entire organ may be involved (the process ordinarily extends proximally). Rarely, the regressive changes may be more marked in the middle or the proximal portion than in the distal third.

In our series there were 39 structures interpreted as involuted false diverticula of the appendix. In 21 cases they were multiple; in 1 case there were 5, and in the others there were fewer. Many of them were found opposite the mesoappendix or at the sides, and it is probable that these false diverticula never attained the size of those involving the mesoappendix. A typical example is shown in figures 8 and 8 *B*. Here the appendix itself shows apparent involution (following old inflammation) and an old perforation of the walls filled with tissues similar to submucosa and subserosa and continuous with these coats of the appendix. The fibrous tissues in the old perforation project well beyond the level of the serosa, and the structure is apparently the remains of a false diverticulum. In all probability it first formed following acute perforative inflammation a long time previously. There then followed progressive development of mucosa, submucosa and subserosa as described, followed by progressive atrophy of all coats, especially the mucosa, with obliteration of the lumen (involution). At about the same time similar changes occurred in the regional appendix itself.

The cause of the progressive atrophy of the walls and obliteration of the lumen of the false diverticulum is probably the same as that which is responsible for similar changes in the appendix itself.

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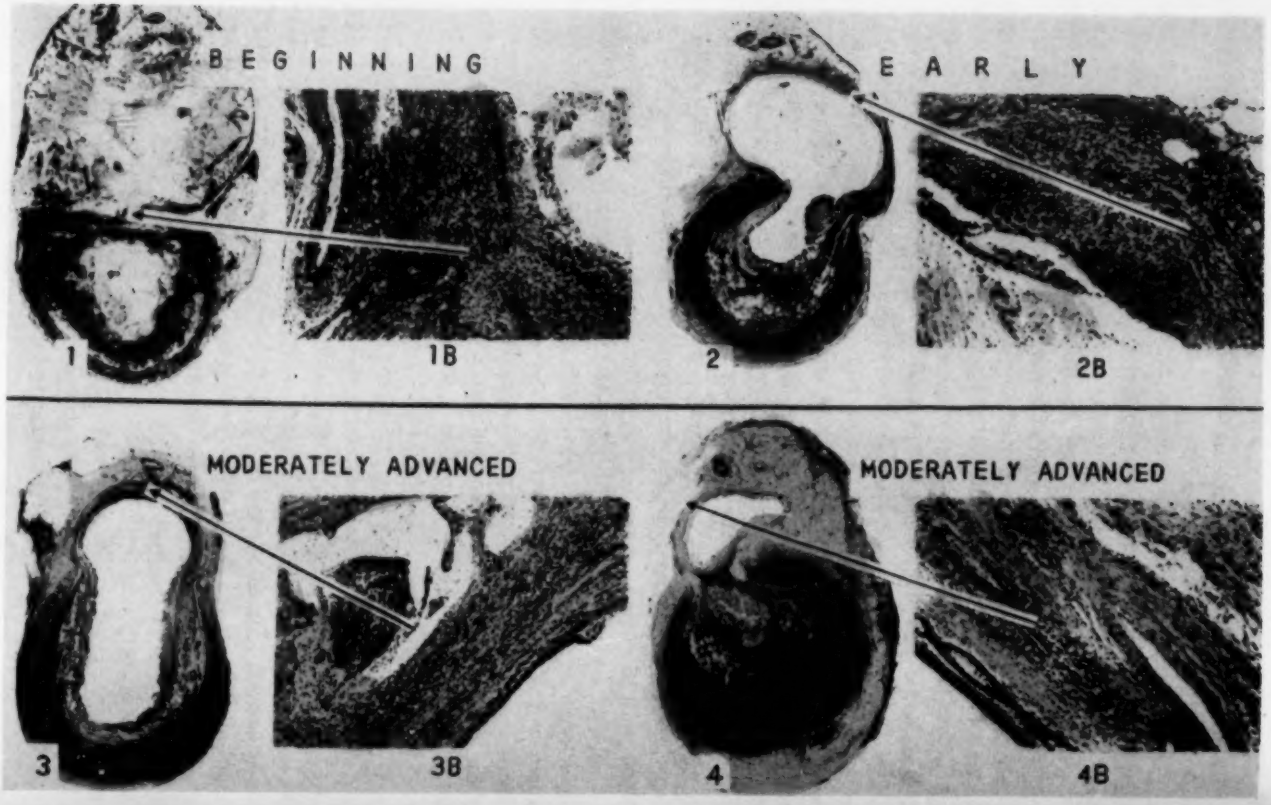
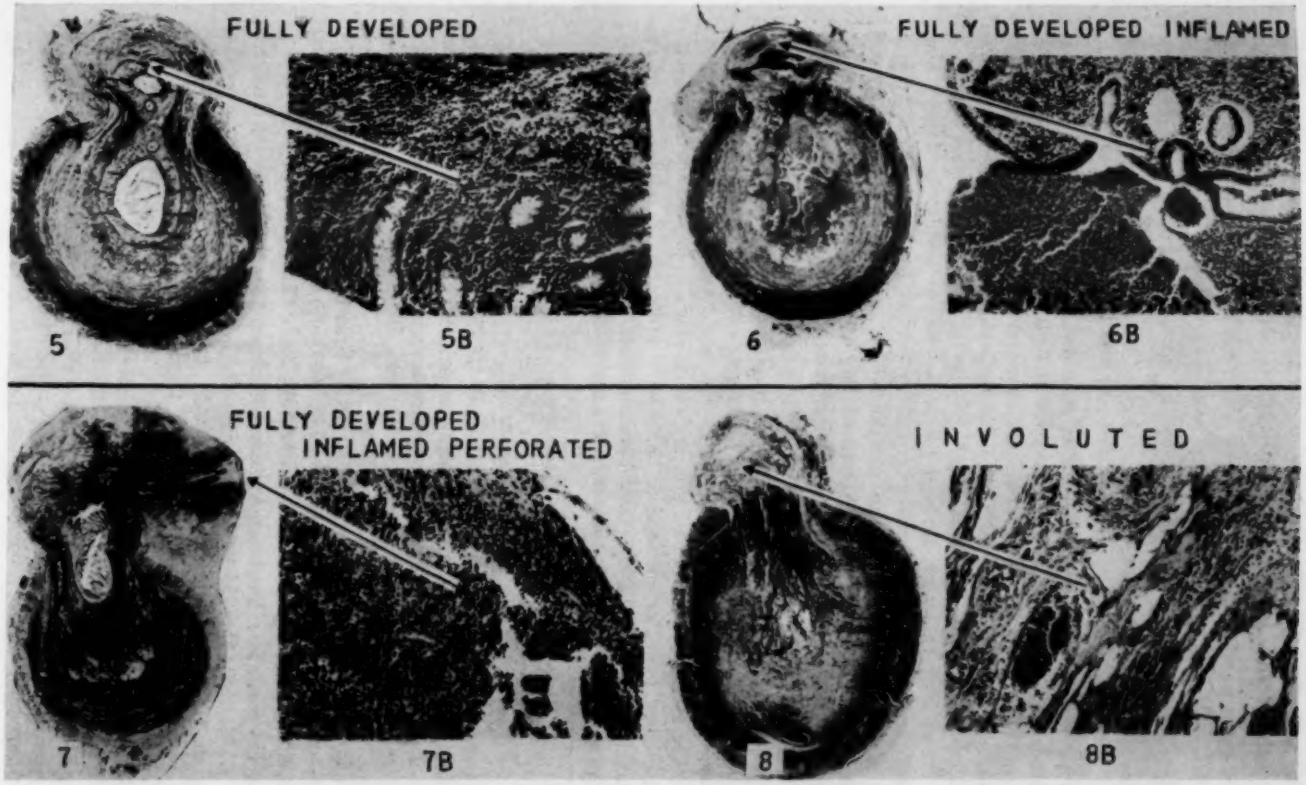
7. Ribbert, H.: Virchows Arch. f. path. Anat. **132**:66, 1893.


BEGINNING
1
1B
EARLY
2
2B
MODERATELY ADVANCED
3
3B
MODERATELY ADVANCED
4
4B



Developmental, inflammatory and regressive changes of false diverticulum formation of the appendix. Figures 1 to 8 show very low magnifications of azocarmine-stained sections; figures 1 *B* to 8 *B*, low magnifications of hematoxylin and eosin–stained sections taken from the region opposite the perforation.

Whereas many of the moderately advanced and fully developed false diverticula in our series were inflamed, the involuted false diverticula were infrequently inflamed (4 of 39).

In cases in which small and very small firm nodules projected beyond the level of the serosa, difficulty was experienced in differentiating involuted false diverticula from areas of healed perforative appendicitis.

HISTORICAL REVIEW

Many early pathologists believed that false diverticulum of the appendix was brought about by herniation of the mucosa through preformed or acquired apertures in the muscle. The only preformed apertures are those regional to vessels and nerves passing through the wall of the appendix (most commonly along the mesoappendical border but occasionally elsewhere). These sites were looked on as areas of least resistance, through which the mucosa herniated. Many[8] have believed these areas of least resistance could be further weakened by distention of the regional blood vessels, as in cardiac failure, which would favor mucosal herniation. Klebs[8a] and Chase[8k] believed they could also be weakened further by atrophy of adipose tissue about the blood vessels, especially in previously obese people.

The acquired apertures were those due to atrophic, degenerative or inflammatory changes in the muscle wall.[9] The mucosa, in cases in which there were preformed or acquired apertures in the muscle, was supposedly caused to herniate because of increased intraluminal pressure due to stasis in or stenosis of the lumen and increased muscular contraction. Krabbel[8d] proposed the theory that in the appendix, as elsewhere in the gastrointestinal tract, traction on the external surface by adhesions may be responsible for some of the diverticula.

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8. (*a*) Klebs, 1868, cited by Hansemann. (*b*) Hansemann, D.: Virchows Arch. f. path. Anat. **144**:400, 1896. (*c*) Graser, E.: München. med. Wchnschr. **46**:721, 1899. (*d*) Krabbel, M.: Beitr. z. klin. Chir. **80**:121, 1912. (*e*) Mündt, R.: Ueber Veränderungen der Muskelwand des Wurmfortsatzes, Pathologisch-anatomisch Arbeiten Herren Geh. Medicinalrath Dr. Johannes Orth zur Feier seines 25jährigen Professoren-Jubilaüms gewidmet von den Göttinger Assistenten, Schüleren und Freunden, Berlin, A. Hirschwald, 1903, p. 453. (*f*) Corning, E.: Albany M. Ann. **26**:816, 1905. (*g*) Kline, L. B.: Mil. Surg. **77**:275, 1935. (*h*) Heschl: Wien. med. Wchnschr. **30**:540, 1880; cited by Seelig.[9g] (*i*) Konjetzny, G. E.: München. med. Wchnschr. **56**:2251, 1909. (*j*) Stout, A. P.: Arch. Surg. **6**:793, 1923. (*k*) Chase, W. H.: Canad. M. A. J. **17**:416, 1927.

9. (*a*) Edel, M.: Virchows Arch. f. path. Anat. **138**:347, 1894. (*b*) Beer, E.: Am. J. M. Sc. **128**:135, 1904. (*c*) Aschoff, L.: Verhandl. d. deutsch. path. Gesellsch. **1**:246, 1904; cited by Oberdorfer.[9f] (*d*) Maaløe, C. U.: Histopathologiske studier over processus vermiformis, Copenhagen, Jacob Lund, 1908; cited by Collins.[2] (*e*) Lejar and Ménétrier: Rev. de chir. **30**:469, 1904. (*f*) Oberdorfer, S.: Ergebn. d. allg. Path. u. path. Anat. **13**:527, 1909. (*g*) Seelig, M. G.: Ann. Surg. **44**:78, 1906. (*h*) Villar, M.: Bull. Acad. de méd. **79**:175, 1918. (*i*) Herb, I. C.: Tr. Chicago Path. Soc. **7**:94, 1907. (*j*) von Brunn, M.: Beitr. z. klin. Chir. **46**:67, 1905. (*k*) Weinberg, M.: Résumé des lésions histologiques des formes communes de l'appendicite, Thesis, Paris, no. 647, 1898.

As interpreted recently by Collins,[2] who studied 30 cases, false diverticulum formation "originates secondary to a preceding appendicitis which has left in its wake stenosing scars that cause varying degrees of lumen occlusion. The efforts of the muscularis of such an appendix to empty its lumen commonly lead to marked increase of the intraluminal pressure which in turn causes, occasionally, the herniation of the mucosa out through areas of lessened resistance in the muscularis which are the vascular hiati. Thus, the walls of the acquired or false appendiceal diverticulum are only composed of mucosa, some loose areolar tissue and the serosa."

In contrast to this view is that of a number of observers, beginning with Letulle and Weinberg,[5] that false diverticulum formation results when an ulcerative lesion of the wall is followed by mucosal ingrowth and development. The inflammatory and reparative changes of the process were adequately presented by von Brunn [9] after a study of 8 cases. There has been no previous mention of involution of false diverticula of the appendix. Such diverticula, however, were apparently interpreted as cicatricial (Letulle, Weinberg [5]) in character or as representing neuroma of the appendix (Dordick [6] and others).

CLINICAL SIGNIFICANCE OF FALSE DIVERTICULA

Collins [2] stressed the clinical significance of false diverticulum of the appendix as follows: "It is becoming increasingly more apparent that diverticula of the vermiform appendix should merit more than mere academic interest on the part of physicians. Acute inflammation occurring in an appendix containing such diverticula is commonly followed by rapid perforation through the diverticulum with a subsequent generalized peritonitis after only a very few hours of atypical abdominal signs and symptoms." The fact that about 29.6 per cent of the false diverticula in our series were acutely inflamed (table) and that quite a number of these were perforated likewise indicates that the incidence of inflammation and perforation of the false diverticulum of the appendix is much greater than that of inflammation and perforation of the appendix itself and that therefore perforative appendicitis with false diverticulum formation predisposes to perforative diverticulitis. It may be because of stasis in the false diverticulum due to the absence of muscle in the wall and because of the very thin wall of the false diverticulum that inflammation, perforation and regional acute peritonitis of the false diverticulum occur more frequently than does the same combination of changes in the appendix itself, and therefore a patient having an appendix with a false diverticulum is in greater danger than one without this complication.

In the majority of cases in our series the past history of the patient with a false diverticulum of the appendix revealed evidence of a previous attack of perforative appendicitis (17 of 31 cases with complete histories). In the 14 cases without symptoms there was apparently focal appendicitis with small perforations into the mesoappendical fat without involvement of the regional peritoneum. No deaths resulted in this series.

The following are representative clinical histories:

BEGINNING FALSE DIVERTICULUM

CASE 1.—A white boy aged 11 years complained of pain in the abdomen for twelve hours. In the beginning the pain was diffuse, sharp and cramplike; the patient vomited several times. Several hours later the pain became localized to the right lower quadrant. There had been no previous attacks of pain in the abdomen. There was marked localized tenderness in the right lower quadrant of the abdomen. There were marked abdominal spasm, rigidity and rebound tenderness. The temperature was 102.3 F.; the leukocyte count, 4,850. The diagnosis was acute suppurative appendicitis with beginning rupture in the mesoappendix and regional acute fibrinopurulent peritonitis.

EARLY STAGE

CASE 2.—A colored woman aged 28 years had pain in the side and back and weakness. Two days prior to admission she had severe pain in the right side. It was an aching pain, which started in the epigastrium and shifted to the right lower quadrant of the abdomen. There were frequent gas pains and occasional nausea. There had been no previous attacks of pain in the abdomen. There were severe tenderness, rebound tenderness and rigidity in the right lower quadrant of the abdomen. The temperature was 100 F.; the leukocyte count, 5,050. The clinical impression was one of chronic cholecystitis and cholelithiasis. The final diagnosis was chronic catarrhal appendicitis and multiple false diverticula of the appendix (early and well developed).

FULLY DEVELOPED STAGE

CASE 3.—A white woman aged 32 years complained of attacks of epigastric pain for five or six years. The patient first began having attacks of epigastric pain, which were not very severe and at times were associated with nausea. In the past eight months attacks became more severe and more frequent, some lasting as long as five days. The past history was essentially irrelevant. The physical findings were essentially negative except for vague tenderness over McBurney's point. The temperature was 98.7 F.; the leukocyte count, 7,200. The clinical impression was that of chronic appendicitis (asymptomatic interval). The final diagnosis was fully developed false diverticulum.

FULLY DEVELOPED, INFLAMED STAGE

CASE 4.—A white woman aged 38 years had pain in the right lower quadrant of the abdomen. About fifteen hours prior to admission she was seized with a sudden sharp pain in the epigastrium, which spread to the umbilicus and later became localized to the right lower quadrant. About six hours after the onset she took a mild laxative (milk of magnesia) and soon after had a bowel movement. Following this, the pain became more severe. She had had two similar attacks, not as severe as this one. One attack occurred three months and another four months before. There was considerable rigidity of the abdomen, especially over the right lower quadrant. There were moderate distention, rebound tenderness and pain on palpation. The temperature was 101 F.; the leukocyte count, 12,000. The diagnosis was acute suppurative and ulcerative appendicitis with acute suppurative inflammation of a fully developed false diverticulum, and acute suppurative periappendicitis.

INVOLUTED STAGE

CASE 5.—A colored woman aged 25 years had abdominal pain. She had had pain in the right lower quadrant for a year. The pain was worse when she was on her feet and working. About ten days before, she became acutely ill with nausea, vomiting, pain in the right lower quadrant and back. She had dysuria and nocturia. The last menstrual period lasted ten days, and the flow was excessive. She had had leukorrhea for one and a half years. One year before, she had right-sided abdominal pain and was quite ill. This condition was diagnosed by a physician as acute appendicitis. Nothing was done at that time. The patient was poorly nourished and appeared ill. The abdomen was rounded and rigid. The rigidity was more marked in the right lower quadrant, with spasm and peritoneal tenderness. On vaginal examination a soft tender mass was palpable in the right side of the pelvis. The temperature was 98.7 F.; the leukocyte count, 8,500. The clinical impression was that of pelvic abscess and chronic pelvic inflammatory disease. The final diagnosis was subacute salpingitis and involuted false diverticulum appendix.

INVOLUTED STAGE

CASE 6.—A white man aged 20 years experienced sensations of fulness in the epigastrium. The complaint started three months before examination. It had no relation to meals, though nausea was sometimes noted after meals. There was no pain or belching. There was a moderate degree of tenderness over McBurney's point but no rigidity or spasm. The temperature was 98.8 F.; the leukocyte count, 7,000. The clinical impression was that of chronic appendicitis. The final diagnosis was involuted false diverticula of the appendix.

SUMMARY

From a study of 85 false diverticula in 49 appendixes it appears that false diverticulum formation in the appendix follows acute perforative inflammation, usually along the mesoappendical border and usually in the distal third of the organ. The inflammatory process extends through the appendical wall and causes necrosis, liquefaction and cavitation of the regional adipose tissue. When the inflammatory process subsides, there is a growth into the cavity in the fat, and subsequent development there of mucosa, submucosa and subserosa derived from these coats regional to the area of perforation. Although much smooth muscle in the originally inflamed area of the appendix is destroyed, there is no appreciable regeneration of this tissue nor any extension of it into the wall of the false diverticulum. The false diverticulum of the appendix, with no muscle in the wall, is easily distinguished from the very rarely observed true diverticulum, with all coats present. At any stage in its development the false diverticulum may become inflamed and may occasionally become perforated. Not infrequently there is gradual atrophy of the wall of the false diverticulum, especially of the mucosa, a process of involution, just as in the appendix itself.

The fact that about 29.6 per cent of the false diverticula in our series were acutely inflamed (table) and that quite a number of these were

perforated likewise indicates that the incidence of inflammation and perforation of the false diverticulum of the appendix is much greater than that of inflammation and perforation of the appendix itself and that therefore perforative appendicitis with false diverticulum formation predisposes to perforative diverticulitis. It may be because of stasis in the false diverticulum due to the absence of muscle in the wall and because of the very thin wall of the false diverticulum that inflammation, perforation and regional acute peritonitis of the false diverticulum occur more frequently than does this process in the appendix itself, and therefore the danger to a patient having an appendix with a false diverticulum is greater than that to one without this complication.

PATHOLOGIC ANATOMY OF CHRONIC ULCERATIVE CECITIS: A SPONTANEOUS DISEASE OF THE RAT

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In a previous communication (Jones and Stewart [1]) a brief report was made of a spontaneous disease in the laboratory rat. The disease is characterized by chronic ulcerative cecitis, chronic lymphangitis, obstructive lymphedema and lymphoid hyperplasia of the nodes of the mesentery. It was first encountered in a colony of rats during attempts to produce tumors by intrahepatic implantation of a carcinogenic hydrocarbon (Jones, Rothman and Shear [2]). Subsequently, its occurrence in other colonies and other strains of rats was established. Since the publication of our previous report we have found in the literature scattered references to the occurrence of the lesions of chronic cecitis in rats under observation for various purposes. However, no more detailed description of this disease has apparently been made, and therefore an account of the pathologic anatomy of the condition and of its development is presented.

MATERIALS AND METHODS

Postmortem examinations were performed on approximately 300 rats from different colonies, ranging in age from birth to 3 years. Most of these animals were of a hooded buff strain from the stock of the Fearing Research Laboratory, Free Hospital for Women, Brookline, Mass. This strain was originated by crossing a Wistar rat with an English wild rat. Other rats in which the disease was studied were obtained from colonies located in the Cancer Investigations Laboratory, United States Public Health Service, Harvard University (Wistar and hooded buff strains) and the National Institute of Health, Bethesda, Md. (Wistar, Buffalo and Yale strains). Also studied were some affected animals of the Wistar strain obtained from a local dealer in the Washington, D. C., district. The animals had been maintained on various diets in the different laboratories, consisting

1. (*a*) Jones, B. F., and Stewart, H. L.: Pub. Health Rep. **54**:172, 1939. (*b*) Stewart, H. L., and Jones, B. F.: Arch. Path. **28**:758, 1939.

2. Jones, B. F.; Rothman, A. J., and Shear, M. J.: Unpublished data.

generally of Pratt's dog chow,[2a] Purina dog chow [2b] or the special Wistar dry ration for the rat [2c] prepared at the Wistar Institute, in Philadelphia. These diets were supplemented by certain other constituents, including bread and milk, lettuce, cabbage, carrots, boiled egg and cod liver oil. Water was allowed freely at all times. No attempt was made to control the diet from the point of view of the development of the cecal lesion. The majority of the postmortem examinations were made on animals immediately after ether asphyxiation; the remainder were made on animals that died from spontaneous causes, at varying intervals after death. Two procedures were followed in the fixation of the tissues after evisceration: 1. Cecums were opened and washed and examined in the fresh state, before fixation. 2. In several cases cecums were filled with dilute solution of formaldehyde U. S. P. (1:10) after ligation of the cut ends of the ileum and colon. The fixatives employed included, in addition to the dilute formaldehyde solution, Tellyesniczky's, Zenker's and Bouin's fluid. Most of the tissue was blocked in paraffin, cut at 7 microns and stained with one or more of the following: hematoxylin and eosin, phosphotungstic acid–hematoxylin, eosin–methylene blue, Masson's trichrome stain,[3] methyl violet, Van Gieson's stain and the Ziehl-Neelsen carbolfuchsin stain. Frozen sections of formaldehyde-fixed tissue were treated with sudan IV and nile blue sulfate.

CLINICAL ASPECTS OF CECITIS

The animals in the early stages of the disease were normal in appearance, and weight and appetite were maintained. A certain number of animals presented no symptoms during life but showed lesions at autopsy; in others diarrhea of a mild degree developed early, usually a few days after birth. Diarrhea was observed in animals of the affected colonies at all ages. In the case of adult rats it was sometimes associated with bloody stools. The gravity of this sign is indicated by the fact that animals otherwise normal in appearance were sometimes found dead shortly after blood was noted in their stools.

In general, palpation of the lower part of the abdomen in a 3 month old rat would reveal in the region of the cecum a movable small nodule, measuring approximately 10 mm. in diameter. As a rule, this mass was tough, hard and lumpy; less frequently it was soft and fluctuant. Later a lower abdominal mass was regularly present, and the animal lost weight and hair progressively and became weak and emaciated. The coat was soon in poor condition, and the rat looked and behaved like a sick animal. In some instances, the abdominal mass increased in size to such an extent that the picture became that of an emaciated animal with a greatly distended abdomen. At this stage

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2a. The formula for Pratt's chow is: protein, 20 per cent; fat, 6.5 per cent; fiber, 3.5 per cent.

2b. The formula for the Purina chow is: protein, 23 per cent; fat, 5 per cent; fiber, 4 per cent.

2c. Greenman, M. T., and Duhring, F. L.: Breeding and Care of Albino Rat for Research Purposes, ed. 2, Philadelphia, Wistar Institute of Anatomy and Biology, 1931, pp. 45-62.

3. Masson, P.: J. Tech. Methods **12**:75, 1929.

rolling the mass between the fingers sometimes elicited a crepitant sound. This sign was due to the presence of calcified material in the wall of the cecum. Several rats died with massive hemorrhage into the cecum and large intestine, and one, with acute diffuse peritonitis. One animal died with a large ulcer of the abdominal wall, to which the ulcerated cecum was adherent. In some affected rats anemia developed; the leukocyte counts ranged from 8,300 to 23,000 cells per cubic millimeter.

The relatively mild character of chronic ulcerative cecitis in the rat is indicated by the fact that death was usually attributed to mechanical or secondary causes. The contributing causes of death included massive intestinal hemorrhage or chronic anemia from continued loss of blood, chronic malnutrition associated with the diarrhea, or bacteremia. Two affected animals showed lymphosarcoma. A common cause of death to be expected in ulcerative disease of the gastrointestinal tract, namely, acute peritonitis, occurred only rarely.

ANATOMY

The cecum of the normal rat is a rounded pouchlike structure, 2 to 2.5 cm. in length, projecting from the head of the large intestine (fig. 1 *A*). At a little over 2 cm. from its blind extremity it receives the terminal part of the ileum; in the same region, near the tumid white ring of the ileocecal valve is the beginning of the large intestine. The cecum is generally found somewhat to the right in the animal's body, but free and not bound down to the right iliac fossa as in man. It is of moderate size and simple in form, characteristics which are correlated in this order with rooted tuberculous molars and nonexclusive subsistence on a vegetable diet. The cecum is divided into an apical portion (appendix) and a basal portion, which is the cecum proper. The appendix is a direct continuation of the cecum, and externally there is only a poorly marked constriction dividing the two. In some animals it is impossible by external inspection to determine the limits of these structures. The apical, or appendical, portion of the cecum contains aggregated masses of lymphoid tissue whereas the basal portion, or true cecum, is free of lymphoid nodules.

The cecum and ascending colon of the rat have no mesentery of their own but are connected with the posterior abdominal wall by the long mesentery of the small intestine, a reflection of which binds the caudal portion of the ileum to these portions of the large intestine. Situated in this ileocolic mesentery is a chain of lymph nodes, which begins near the ileocecal junction and extends craniad, following the course of the superior mesenteric artery and lying for a part of the distance near and parallel to the ascending colon.

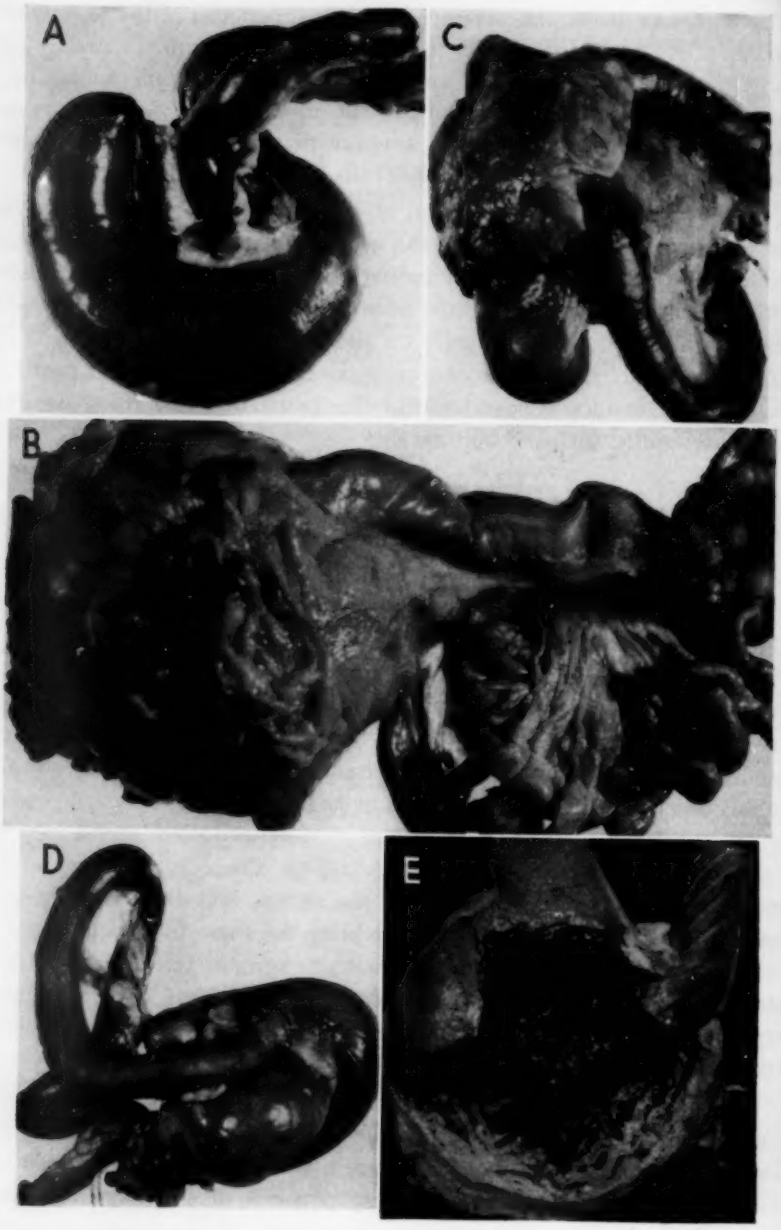

Figure 1

(See legend on opposite page)

The anatomy of the lymphatic system in the adult rat has been described by Job,[4] and the cytologic character of the lymph node, by Kindred.[5] On injection of dyes into the intestinal walls, numerous small vessels are shown collecting into larger and larger vessels until these finally join the great intestinal lymph vessel of the mesentery. This main channel follows the large intestine from the appendix to the anterior upper part of the abdomen. Here there is a single large intestinal node, which lies just proximal to the cisterna chyli and marks the branching of the large intestinal lymph vessel. From this point, one branch goes to the cisterna chyli and the other to the portal vein; the latter branch, although not constantly present in all animals, is still fairly common. From the cisterna chyli the thoracic duct leads dorsolaterally along the superior vena cava and left innominate vein to its juncture with the venous system in the jugulosubclavian district.

4. Job, T. T.: Anat. Rec. **9**:447, 1915.
5. Kindred, J. E.: Am. J. Anat. **62**:453, 1938.

EXPLANATION OF FIGURE 1

A, cecum, appendix and adjacent colon and ileum from a normal rat for comparison with the other gross specimens. The normal specimen was filled with dilute solution of formaldehyde U. S. P. after ligation of the severed ends of the intestine. There is no distinct line of demarcation between the cecum and the appendix. Photograph of gross specimen.

B, intestines and mesentery. The cecum (left) is enlarged, rounded and covered by thickened peritoneum. There is a mass of lymph nodes adherent to the cecum below its junction with the colon, and smaller nodes may be seen in the mesentery paralleling the colon. Photograph of gross specimen.

C, cecum and appendix (left), ileum and colon (right), fixed in the distended state. The cecum is covered with adhesions. The appendix (lower left) is sharply demarcated from the cecum and relatively free of adhesions. The mesentery is thickened and conceals a group of enlarged cystic lymph nodes in the angle made by the ileum and the colon with the cecum. Photograph of gross specimen.

D, cecum, appendix adjacent colon and ileum and mesenteric lymph nodes. The specimen was filled with fixing fluid after ligation of the severed intestines. The mass of lymph nodes visible in the foreground are enlarged and cystic. To the right of the nodes and above is the ileocecal junction, and above this point, the atrophied cecum and appendix, extending from right to left behind the terminal portion of the ileum. The cecum and appendix together measure only 15 mm. in length. Photograph of gross specimen.

E, interior of cecum; appendix (left), ileum (above) and colon (right). The specimen was hardened in fixing fluid and split in two equal halves, one of which is shown in the illustration. The mucosal surface of the cecum is covered with laminated, shaggy, necrotic material containing deposits of calcium. The lesion is sharply confined to the cecum, leaving the mucosa of the appendix, colon and ileum uninvolved. Photograph of gross specimen.

There are two types of lymph nodes in the rat. One type is a single node in which the lymph enters at the periphery and passes through the body of the node to the hilus, where a lymph vessel is formed. The other type is a double node in which two single nodes are bound together in the same connective tissue capsule. There is usually a single node at the juncture of each mesenteric lymph vessel with the main intestinal lymph vessel. A varying number of single lymph nodes also occur in the region of the large intestinal node, and these are all connected with the intestinal lymph vessels. In the region of the appendix there are several lymph nodes, which are usually single. In some instances, however, they are so united as to form one large compound node, 2 or 3 cm. long.

GROSS OBSERVATIONS

The characteristic lesions consisted of cecal ulceration, enlargement of the cecal lymph nodes and changes in the adjacent peritoneum, mesentery and lymph vessels (fig. 1 *B, C, D* and *E*).

The cecum showed discrete small gray subserous and submucous lesions measuring 1 to 2 mm. in diameter in the early stages. These were frequently associated with shallow irregular mucosal ulcers, subserosal ecchymoses, edema and hemorrhage. These early changes progressed to gross alteration in the cecum and adjacent structures. The degree of involvement varied in the different animals studied, without close reference to age. Thus, the process was observed well developed in animals 10 months of age and, on the other hand, it was encountered in an early stage in 1 animal at 27 months. In the majority of cases, at a late stage the cecum was converted into a dilated, rigid cystlike spheric mass, 30 to 70 mm. in diameter. Sometimes, however, the elongated pouchlike contour of the structure was maintained, even in the presence of an extreme alteration in size. On palpation the cecal mass was not of uniform consistency; firm areas alternated with compressible areas. The rigidity was due to fibrosis of the wall together with deposits of calcium. Atrophy and contraction of the viscus into a small hard globular or stublike appendage, not more than 15 mm. long, occurred in a few cases. In 1 case no vestige of the cecum was found. The lumens of these atrophic specimens were considerably narrowed, the walls thickened and fibrotic, and the mucosa usually totally ulcerated. A striking feature of the gross appearance was the sharp localization of inflammatory and ulcerative lesions to the cecum. The ileum and colon were rarely involved (fig. 1 *E*). When lesions occurred in the intestine adjacent to the cecum, the involvement appeared to be a continuation by extension from the cecal focus. The appendix usually showed gray lesions in the submucosa and occasionally

ulceration. The cecal ulcers were covered by a pseudomembranous necrotic exudate, sometimes containing calcium deposits. In some specimens there was a more or less complete shell of calcareous material replacing the cecal wall. In a number of rats dying suddenly, the lumen of the ceum was filled with clotted blood, and there was blood from recent hemorrhage in the adjacent large intestine. In spite of great alteration in the size and shape of the cecum, there were few if any signs of intestinal obstruction.

Peritoneal adhesions developed early. They were found uniting the cecum with the wall of the pelvis and abdomen and with the urinary bladder, testes, coils of small intestine, stomach, spleen and cecal lymph nodes. In a few cases there was almost total obliteration of the peritoneal cavity by fibrous adhesions. In 1 case the entire visceral and parietal peritoneum was covered by a coating of fibrin, and masses of soft gray fibrinous exudate were floating in a large amount of free fluid in the peritoneal cavity. In this animal perforation of the intestinal wall was not observed. A few small chronic abscesses were observed in the mesentery. The formation of adhesions was preceded by thickening and opacity of the serosa of the affected intestine and adjacent mesentery and the development of discrete and confluent small grayish granular lesions in the peritoneal membranes. The portion of the cecum covered by adhesions corresponded roughly in surface area to that of the mucosal ulceration (fig. 1 *C*). In several cases the uterus was encased in adhesions emanating from about the cecum. One animal showed a cecouterine fistula. In a number of instances there was marked edema of the adhesions that extended between the cecum and the adjacent mesenteric lymph nodes.

Enlargement of the lymph nodes adjacent to the cecum constituted one of the most impressive anatomic features of the disease (fig. 1 *C* and *D;* fig. 2 *A*). It was this group of swollen cystic nodes at the ileocecal junction which was most striking and most apt to attract attention to the condition. These nodes, one to five in number, showed progressively enlargement, edema, lymph stasis, fibrosis and cyst formation. The cystic nodes were tense and translucent and sometimes buried in dense adhesions. All stages in the development of the cystic nodes were observed, from beginning visible dilatation of lymph sinuses to pronounced honeycombing of the entire node with the formation of multilocular cysts (fig. 2 *A*). The larger of these cysts were smooth thin-walled cavities, 1 to 5 mm. in diameter, containing clear fluid, which coagulated in fixatives. Involvement of these lymph nodes was observed in practically all rats showing pronounced chronic cecitis and in some rats in which the cecum was only slightly affected. Usually the lymph nodes in other parts of the body, such as the axillary, inguinal

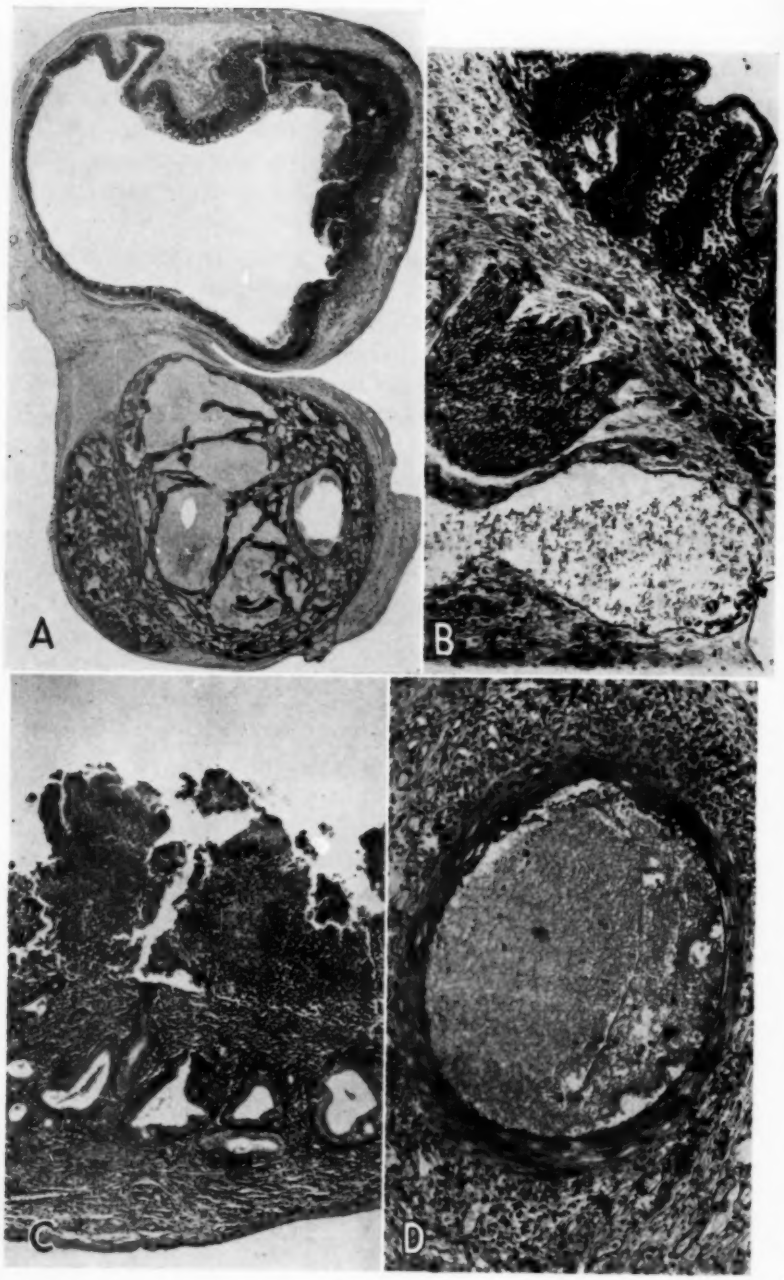

Figure 2

(See legend on opposite page)

and cervical regions, were not involved.[6] In a large percentage of the animals, however, similar changes were also observed in the other lymph nodes of the ascending mesenteric chain. The process diminished in intensity as the distance from the cecum increased. In perhaps a half-dozen cases the lesions described were also present in a group of lymph nodes at the hilus of the lung. This group of nodes was never involved beyond the stage of simple hyperplasia, moderate edema and slight dilatation of lymph sinuses.

Two rats showed lymphosarcoma. One had involvement only of the lymph nodes adjacent to the terminal part of the ileum. In this case a small, shallow, rudimentary-like pouch was the only structure found at the junction of the ileum and colon; it apparently represented the cecum. In the other rat the lesions of lymphosarcoma were more extensive and involved most of the mesenteric and some of the peribronchial nodes. A small ulcer was observed in the tip of the appendix. There were no apparent cystic or fibrotic changes in the lymph nodes of either animal, and the axillary and inguinal nodes showed no visible changes.

In several rats with chronic cecitis and in 3 without intestinal changes the mesentery showed pea-sized abscesses. In a few others suppurative salpingitis occurred. The lungs of a number of the animals showed the well known lesions of chronic bronchiectasis together with small red infarcts. In a few, the liver and spleen contained minute gray miliary nodules. In 1 rat the liver showed large areas of dark gray

6. Other workers have reported to us in personal communications and have shown us specimens illustrating the occasional finding of cystic changes in peripheral lymph nodes of rats and mice.

EXPLANATION OF FIGURE 2

A, cecum and adjacent lymph node. There is a necrotic ulcer involving approximately one fourth of the circumference of the mucous surface of the cecum. The lymph node shows numerous dilated spaces filled with coagulated eosin-staining material. About × 4.

B, cecum. There is a dilated lymph channel in the submucosa. There was no ulceration of the cecum in this specimen, but the submucosa was thick and fibrous, and the mesenteric lymph nodes were enlarged and fibrotic, and showed dilatation of the lymph sinuses and lymph vessels. × 100.

C, cecum. There is ulceration with destruction of the mucosa and musculature. The wall of the cecum is composed of inflammatory cells, granulation tissue and fibrous tissue, in which are embedded remnants of the mucosal glands. The internal surface of the specimen is covered with necrotic exudate. There is nothing specific about the inflammatory reaction. × 50.

D, wall of cecum. A thrombosed artery is surrounded by fibrous tissue and inflammatory cells, which have infiltrated a segment of the vessel wall (above) which is necrotic. × 100.

mottling, due to acute diffuse hepatitis with central necrosis. Aside from limited local extension of the cecal changes, as described, none of the animals showed any involvement of the large or of the small intestine, stomach or esophagus.

MICROSCOPIC OBSERVATIONS

The process was inflammatory in character, and in the various locations in which it was studied, diffuse, focal and nonspecific. The inflammatory areas were vascular, and the infiltrating leukocytes were frequently found in the perivascular tissues. The small focal lesions consisted predominantly of lymphocytes, plasma cells and mononuclear cells, while the large diffuse areas had, in addition, numerous polymorphonuclear leukocytes and eosinophils. Many mononuclear leukocytes were pigmented and vacuolated. The arteries showed chronic periarteritis and endarteritis obliterans, and there were lymphangitis and lymph stasis at all stages of the process. As the condition progressed, the inflammatory cells in the lesions diminished in number, leaving a relatively cell poor but often edematous fibrous granulation tissue in the base of the ulcers, in the adhesions and around the vessels and the lymph nodes.

Cecum.—An infiltration of inflammatory cells into the wall of the cecum was noted in several but not in all young rats examined post mortem at the age of 7, 10, 14, 25 and 90 days. Doubtless, little significance would have been attached to these alterations if attention had not been focused on discovering the earliest manifestations of the condition. The inflammatory foci consisted of 8 to 15 cells (chiefly polymorphonuclear leukocytes, eosinophils, small round cells and mononuclear cells) in the submucosa, musculature and subperitoneal tissue of the cecum, where they appeared to occupy small spaces, possibly lymphatic vessels. The earliest ulcer observed was found in a rat 2 weeks old, which had associated inflammatory changes of mild character in the mucous membrane. This was an exceptional case, however, since all other animals showing frank ulcers were 3 months of age or older.

Most of the cecums examined presented far advanced stages of the process, with fibrosis, extensive ulceration and destruction of the mucosa and musculature (fig. 2 *A, B* and *C*). The ulcers, which appeared to originate in the inflammatory areas, were sharply demarcated. The base was composed of granulation tissue, while the surface was covered with necrotic inflammatory exudate and clumps of micro-organisms. The inflammatory reaction in the ulcerated cecums was more intense, with a higher percentage of polymorphonuclear leukocytes, than that in the nonulcerated specimens. The areas of ulceration were often limited to

the mucosa and submucosa but in some cases extended through the musculature into the subperitoneal tissue. In several cases the base of the ulcer extended through adhesions into adjacent structures, including lymph node, pancreas and anterior abdominal wall. As a rule, perforation of the wall of the cecum occurred too slowly to produce rupture with generalized peritonitis. Large areas in the wall of the cecum were replaced by fibrous tissue. In 1 adult animal showing only a minimal inflammatory lesion in the wall of the cecum there was marked dilatation of the lymph vessels in the submucosa (fig. 2 *B*); the adjacent lymph nodes showed more fibrosis than expected from the inflammatory lesion present in the cecum at autopsy. In general, the lymph vessels in the submucosa of the cecum, although occasionally distended, did not show engorgement to the degree or with the frequency to be expected in view of the marked lymph stasis in the mesenteric lymph nodes. On the peritoneal surface opposite the ulcers in most specimens were small, localized inflammatory and necrotic plaques, composed of fibrin and leukocytes. Isolated and altered remnants of the mucosa sometimes survived among the inflammatory cells in the presence of otherwise extensive destruction of the wall of the cecum (fig. 2 *C*).

In a number of rats the cecum was found on gross inspection to be small and atrophic (fig. 1 *D*), as mentioned in a foregoing paragraph. The wall was markedly thickened and the lumen considerably reduced. These contracted cecums lacked the laminated internal surface described in the dilated specimens; instead the lumen was smooth and bordered all around by granulation tissue, which replaced the musculature wholly or in large part.

The arteries in the wall of the cecum, in the mesentery and in the pericecal adhesions showed acute and chronic arteritis with reduction in the caliber of the lumen (fig. 2 *D* and fig. 3 *A* and *B*). The ratio of lumen to wall in many of these vessels was 1:6; many vessels were totally obliterated. The inflammatory changes in the periarterial tissue consisted of slight to marked perivascular round cell and leukocytic infiltration with invasion and destruction of the vessel wall and thrombosis. These lesions were focal in distribution, causing minute nodular swellings along the course of the blood vessel, resembling those of periarteritis nodosa.

Lymph Nodes and Lymph Vessels.—The lymph nodes in the mesentery near the cecum showed, singly or in combination, hyperplasia, atrophy, focal necroses, fibrosis and dilatation of the lymph sinuses and local lymph vessels. Primary inflammatory hyperplasia and lymph stasis were observed in the nodes, followed by cystic dilatation of the sinuses, fibrosis, atrophy and disappearance of the cellular elements (fig. 2 *A;* fig. 3 *C* and *D*). The primary changes in the lymph nodes

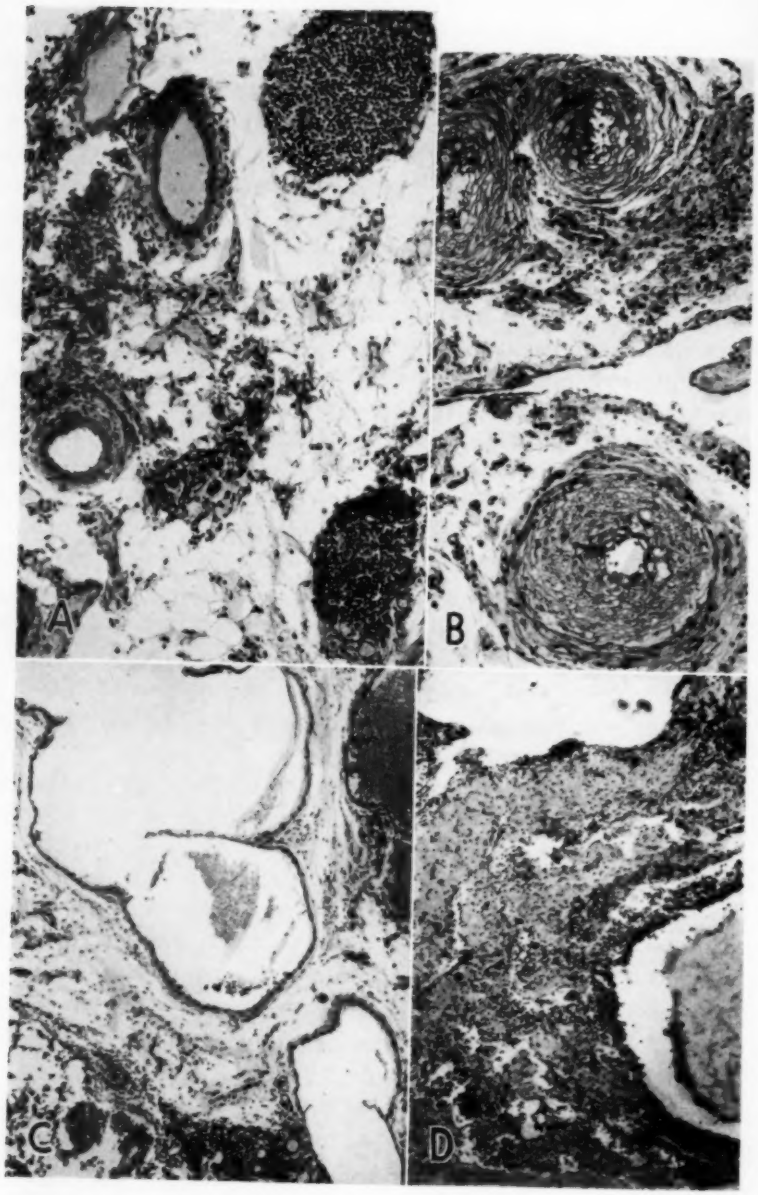

Figure 3

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were studied in immature rats as well as in adult rats in which the cecal process was in an early stage. The lymph follicles in some rats under 1 month of age were hyperplastic; the sinuses were slightly dilated, and there was a sparse infiltration of mononuclear leukocytes and eosinophils. Cecal ulceration in a rat 3 months old was accompanied by follicular atrophy of the adjacent nodes and more pronounced cellular infiltration with many plasma cells. The sinuses of these nodes were dilated and contained many cells, chiefly mononuclear cells and eosinophils. In this process the afferent lymph vessels also became dilated. Collagen was deposited in localized areas in the node or diffusely between the cells. As the node became progressively fibrotic and cystic, the lymphoid cells diminished in number. In the advanced stages of this process the general topography of the node was greatly altered and in places germinal centers were no longer recognizable (fig. 3 *D*). There was fibrous thickening of the capsule and trabeculae, and many lymph nodes with thickened capsules were embedded in the dense fibrous tissue of the mesentery and surrounding adhesions. The degree of sinusoidal dilatation varied considerably in the different nodes and in different parts of the same node. The dilated lymph sinuses contained lymphocytes, vacuolated and pigmented mononuclear cells, fibrin and lightly stained homogeneous material. Dilated lymph vessels were identified in the connective tissue around the capsules of the nodes, in the mesentery and adhesions and in the wall ot the cecum. Some lymph vessels were thin walled, while others showed perilymphangitis and fibrosis. Repeated efforts by appropriate histologic methods failed to demonstrate lipoidal substances in the lymph sinuses.

Lymph nodes from all the various regions of the body were examined histologically. A number of small mesenteric nodes showed dilatation

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EXPLANATION OF FIGURE 3

A, mesentery and pericecal adhesion. There are focal areas of round cell infiltration and perivascular fibrosis. × 75.

B, mesentery and pericecal adhesion. Three markedly thickened arteries with narrowed lumens are embedded in the fibrofatty tissue, which is infiltrated with inflammatory cells. × 100.

C, mesenteric lymph node and surrounding mesentery adjoining cecum. A portion of the node included at the lower margin of the illustration shows early dilatation of lymph sinuses and atrophy of the follicles. Emerging from the hilus of the node is a dilated varicose lymph vessel, which continues upward across the field. × 50.

D, mesenteric lymph node near cecum. Lymph sinuses, which are dilated and which contain coagulated material, may be seen in part above and on the right of the illustration. The portion of the node between the dilated sinuses is fibrotic and hyalinized. × 50.

of the sinuses and chronic lymphadenitis and lymphangitis, identical in character with that already described but of less marked severity. Some of the small arteries in sections from these nodes were also thickened. The peritracheal lymph nodes in a few rats showed in lesser degree the sinusoidal dilatation, cellular hyperplasia and other changes observed in the mesenteric lymph nodes.

Liver and Spleen.—In most animals the liver and spleen showed little or no change. In some, however, there was degeneration to a variable degree in the former and cellular hyperplasia in the latter. Sections of one of the livers showed fibrous thickening of the portal areas with infiltration by round cells and an organizing thrombus in a large vein. In a number of the animals the spleen, liver and lymph nodes contained small focal inflammatory lesions identical in appearance with the focal necroses in human typhoid fever.

COMMENT

The course of this disease is slowly progressive and chronic. Extensive involvement of the cecum and of the adjacent lymph nodes and mesentery may develop before marked symptoms occur. The most advanced lesions have been found in very old rats that had survived for months, although emaciated and bearing each a palpably large abdominal mass. In certain rats exceptions to the usual course of the disease have appeared; some animals more than 2 years old have been found to exhibit only early lesions at autopsy, while some less than 1 year old have shown far advanced lesions. A considerable variation was found in the incidence and severity of the condition in the same and in different stocks of animals.

An effort was made to identify the lesions of this disease with those of the diseases known to occur spontaneously in the rat. The features of the well known granulomas were not reproduced. Giant cells occurred rarely in the inflammatory process, and tubercle formation was not observed. Pseudotuberculosis (infection with Bacillus pseudotuberculosis rodentium [Pfeiffer]),[7] a rare disease in the laboratory rat, has clinical and pathologic features so distinctive that differentiation from chronic cecitis is not difficult. Ulceration of the intestinal mucous membrane does not regularly occur in pseudotuberculosis, and, according to Jaffé,[8] the involvement of lymph nodes is more generalized, with no special localization to the region of the cecum. Characteristic focal

7. Haas, V. H.: Pub. Health Rep. **53**:1033, 1938. Topping, N. H.; Watts, C. E., and Lillie, R. D.: ibid. **53**:1340, 1938.

8. Jaffé, R.: Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere: Kaninchen, Meerschweinchen, Ratte, Maus, Berlin, Julius Springer, 1931.

lesions of the spleen, liver and lymph nodes occur, from which B. pseudotuberculosis rodentium can be recovered. Differentiation from plague can be made on the basis of the absence of buboes, the nonhemorrhagic character of the lymph node lesion in chronic cecitis and the extreme susceptibility of the white rat to infection with the plague pasteurella. Whereas cecitis in the albino rat is a low grade, chronic disease, plague in this animal is a more rapidly advancing, fatal disease. The lymph node lesions of chronic cecitis are usually restricted to the cecal region, contrary to the observation in pseudotuberculosis and plague, in which the axillary and inguinal nodes are usually involved.

Meyer [9] stated that paratyphoid infection in the rat, either in an epidemic form with a high mortality (75 to 100 per cent) or as a latent infection, has been described by a number of workers. In the acute form, diarrhea and the formation of bloody crusts around the eyes are quite characteristic. At autopsy engorgement of the spleen, multiple focal necroses (not specific for Gärtner infection), enlargement of lymph nodes and of Peyer's patches and a blood-tinged intestinal content are rarely absent. The chronic form may show few or no lesions, according to this author. Buchbinder, Hall, Wilens and Slanetz [10] obtained cultures of Salmonella enteritidis from rats dying of epidemic diarrheal disease. They stated that the salmonella infection was enzootic in this colony and that the colony showed a low mortality and few or no external signs of the disease. Infected rats were found to excrete paratyphoid organisms for relatively long periods. At postmortem examination animals excreting paratyphoid bacilli were found to exhibit ulcerative cecitis associated with cystic lymphadenitis, a pathologic picture essentially the same as that we have described in the preceding pages. It was found that naturally infected adult rats which excreted paratyphoid bacilli for relatively long periods could be divided into two groups on the basis of "continuous" and "intermittent" excretion of the organisms. The extent of the lesions observed post mortem roughly paralleled the number of bacteria excreted. The morbid anatomic changes of this so-called carrier condition in rats were recognized by Buchbinder and co-workers as different in character and degree from those previously described by many authors for acute fatal paratyphoid infection. In this report the authors did not submit conclusive evidence to prove that the lesions of chronic cecitis described were attributable solely to paratyphoid infection. They reported, however, the simultaneous occurrence of the intestinal lesions in association with excretion of paratyphoid

9. Meyer, K. F.: Communicable Disease of Laboratory Animals, in Jordan, E. O., and Falk, I. S.: The Newer Knowledge of Bacteriology and Immunology, Chicago, University of Chicago Press, 1928, chap. 45, pp. 636-637.

10. Buchbinder, L.; Hall, L.; Wilens, S. L., and Slanetz, C. A.: Am. J. Hyg. **22**:199, 1935.

bacilli in the feces, the recovery of paratyphoid organisms from the organs of affected animals and the rough parallel between excretion of organisms and cecal lesions. Jones and Donahue [11] isolated a strain of Salmonella enteritidis from a number of rats affected with lesions of chronic cecitis but in a few trials were unable to reproduce this disease. Buchbinder and others did not report production of chronic cecitis by administration of the salmonellas to unaffected animals. The literature is replete with reports indicating the widespread occurrence of salmonella infection in laboratory and wild rats. While S. enteritidis may be related to chronic cecitis in some way, it is our opinion that evidence is lacking at present to establish infection with this organism as the cause of chronic ulcerative cecitis in the rat.

Lynch [12] described ulcers of the cecum produced in wild rats by feeding them a virulent strain of Endamoeba histolytica. Woolsey and Black [13] reported the occurrence of intestinal lesions in guinea pigs on a vitamin D–deficient diet after these animals had been given a dose of fusospirochetal organisms obtained from lesions of pyorrhea alveolaris. Reichert and Mathes [14] produced chronic lymphedema of the bowel in dogs by injecting corrosive material into the mesentery and subserosal lymph vessels. When bacteria were injected in conjunction with the lymphatic injection of corrosive material, a greater thickening of the intestinal wall occurred. These investigators suggested that there is a close resemblance between the pathologic changes of experimental lymphedema which they produced in the dog and those of regional ileitis in man.

The relatively sharp localization of the lesions of chronic cecitis to a comparatively small segment of the intestinal tract of the rat suggests an analogy with regional ileitis in man. According to the published reports, in over 50 per cent of the cases of regional ileitis the lesions occur in the terminal part of the ileum. The lesions of cecitis in the rat are restricted to the cecum and a portion of the appendix and only rarely extend into the colon or ileum for a short distance. The anatomic changes in the early stages of both diseases may be parallel. The affected intestine is found thickened, edematous and covered with reddened peritoneum. There are usually lymphadenitis and lymphedema of the regional mesentery. The primary inflammatory process in the wall of the intestine in both conditions proceeds so slowly that perforation and consequent diffuse peritonitis seldom occur. The chief point of difference between the two conditions is the marked cystic alteration of the

11. Jones, B. F., and Donahue, D. D.: Unpublished data.

12. Lynch, K. M.: J. A. M. A. **65**:2232, 1915.

13. Woolsey, F. M., and Black, J. R.: Arch. Path. **28**:503, 1939.

14. Reichert, F. L., and Mathes, M. E.: Ann. Surg. **104**:601, 1936.

lymph nodes adjacent to the cecum in chronic cecitis and the characteristic infiltration of the intestinal granulation tissue by giant cells in regional ileitis. There are also differences in the characteristic outcome of the two diseases. In the human condition, intestinal obstruction may intervene at any time and may be preceded or followed by fistula formation. In the rat, on the other hand, fistula formation is rare. Intestinal obstruction, a regular feature of regional ileitis, rarely occurs in the rat. This is not a fundamental difference between the two conditions but may be attributed to anatomic variations in the two species. The lesions of regional ileitis involve the main pathway of the intestinal tract and hence allow of no alternative passage when inflammation and repair proceed to the point of local obstruction. The stenosing lesions of chronic cecitis, on the other hand, involve the cecum and appendix, structures which in the rat lie to the side of the direct pathway of the stream of intestinal contents.

There are few reports (Willis [15]) of any neoplasm arising in the region of the cecum in rats. Bullock and Curtis [16] published an account of spontaneous tumors which arose in 489 rats of a colony under observation in the course of experimental infestation with Cysticercus fasciolaris. In this study 10 tumors, viz., 7 diagnosed as sarcoma, 1 as "gelatinous carcinoma" and 2 as fibroma, originated in the cecum, which was the only portion of the large intestine giving rise to primary tumors. Seventy-eight of the tumor-bearing animals also had sarcoma of the ileocecal and other mesenteric lymph nodes. Slightly over half of the rats with mesenteric tumors were shown to have cecal ulceration. Bullock and Curtis stated that the latter tumors, diagnosed as sarcoma, arose in lymph nodes draining areas prone to ulceration and subject to inflammatory lesions. We have observed 2 rats with cecal disease which also had lymphosarcoma, involving chiefly the nodes in the cecal area. These findings suggest the possibility of an etiologic relationship between these neoplasms and chronic cecitis.

While there may be an etiologic relationship between certain neoplasms and chronic cecitis, it is also true that the lesions of chronic cecitis may have been mistaken for neoplasms. Roffo [17] reported ulcers and malignant tumors ("fusocellular sarcoma" and "adenomatous neoplastic formations") occurring in the cecums of rats following the latter's ingestion of irradiated cholesterol or irradiated food rich in cholesterol. The ulcerative lesions in the cecums of these animals were associated with inflammatory changes and enlargement of adjacent mesenteric lymph nodes. The author stated that such lesions were never observed

15. Willis, R. A.: J. Path. & Bact. **40**:187, 1935.

16. Bullock, F. D., and Curtis, M. R.: J. Cancer Research **14**:1, 1930.

17. Roffo, A. H.: Bol. Inst. de med. exper. para el estud. y trat. d. cáncer **14**:589, 1937.

in rats fed on the usual bread and milk diet. From the descriptions and the illustrations of these lesions it appears that Roffo's rats exhibited pathologic changes of the cecum similar to those in animals with chronic cecitis. Because of the relatively frequent occurrence of spontaneous cecitis in laboratory rats, particular care should be exercised to avoid confusion of the lesions of this disease with experimentally produced changes.

The literature contains references to the occurrence in rats of lesions typical of chronic cecitis, encountered in the course of experimental and descriptive studies. Smadel and Farr [18] noted cecal lesions in rats in the course of a study of induced nephrotoxic nephritis. Among these lesions were certain vascular changes associated with cecal inflammation, both of which the authors attributed to the experimental treatment. The fact that these lesions as described and illustrated by Smadel and Farr are similar to the vascular and associated cecal changes arising spontaneously in chronic cecitis may cast some doubt on this conclusion. Lynch [12] reported the occurrence of ulcers of the cecum in the course of observations and experiments on wild rats trapped in Charleston, S. C. Ulcers were found in untreated wild rats harboring amebas, which the author considered indistinguishable from Endamoeba histolytica. In other wild rats from the same source cecal ulcers and lymph node changes were noted after the animals had ingested E. histolytica from infected human subjects. The incidence of spontaneous cecal ulceration in wild rats with or without amebas has not been established. On the other hand, it does not appear likely that the lesions of chronic cecitis in laboratory rats can be explained on the basis of amebic infection. C. W. Rees,[19] of the Division of Zoology of the National Institute of Health, has identified Endamoeba muris in the cecal contents of affected animals. It is his opinion that these organisms, which are commonly encountered in the cecum of the rat, were not causally related to the lesions of cecitis.

SUMMARY

The pathologic anatomy of a spontaneous disease in the laboratory rat is described and illustrated. The disease is characterized by chronic ulcerative cecitis, chronic lymphangitis, obstructive lymphedema and lymphoid hyperplasia of the nodes of the mesentery.

18. Smadel, J. E., and Farr, L. E.: Am. J. Path. **15**:199, 1939.
19. Rees, C. W.: Personal communication to the authors.

EFFECTS ON THE BLOOD AND HEMOPOIETIC ORGANS OF THE ALBINO RAT OF REPEATED ADMINISTRATION OF BENZENE

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OMAHA

Since the report given by Selling,[1] in 1916, of the changes induced in the blood and hemopoietic tissues of the rabbit by administration of benzene, the histologic effects experimentally induced by the introduction of this chemical compound have not been reported in detail. In view of the fact that this compound and its derivatives have been given so much prominence as possible etiologic agents in certain blood dyscrasias, particularly malignant neutropenia (Kracke[2]; Damashek[3]), we considered it profitable to investigate its toxic action and to attempt to correlate more exactly the effects encountered in the hemopoietic tissues with the extensive changes noted in the blood picture during the time of the administration of benzene.

MATERIALS AND METHODS

In contrast to most previous investigators, we chose the albino rat as the experimental animal, being careful to select animals ranging in age from 3 to 6 months. They were from a colony which had been maintained for several years and in which the normal range of variation in hemograms and hemopoietic tissues had been established during the course of other investigative work. All were given a standard diet, which included freshly cooked meat, lettuce, carrots, bread and various grains, such as wheat, corn, and oats; this diet was supplemented by cod liver oil twice weekly.

The experimental animals were subjected to daily subcutaneous injections of a mixture of equal parts of chemically pure benzene and pure olive oil, the latter being added to delay the absorption of benzene. In our first group of experiments the dosage was 2 cc. per kilogram daily; in the second group, 3 cc. per kilogram daily, and in the third group, 4 cc. per kilogram daily. As controls in each group, one animal was untreated and another received daily injections of olive oil to

From the Department of Anatomy, University of Nebraska College of Medicine.

1. Selling, L.: Johns Hopkins Hosp. Rep. **17**:83, 1916.

2. Kracke, R. R.: Am. J. Clin. Path. **2**:11, 1932. Kracke, R. R., and Carver, H.: Diseases of the Blood and Atlas of Hematology, Philadelphia, J. B. Lippincott Company, 1937.

3. Damashek, W.: J. A. M. A. **93**:712, 1929.

rule out any possible effects of this substance on the blood or the blood formation. Total erythrocyte and leukocyte counts were made daily on both control and experimental animals during early phases of the experiment; in later stages, at forty-eight hour intervals. Differential leukocyte counts were made routinely, the smears being stained with Wright's stain followed by a 1:20 dilution of Giemsa's stain. Occasionally brilliant cresyl blue was added in order to determine the percentage of reticulocytes. Hemoglobin determinations were made with the Dare hemoglobinometer.

Rats were killed at regular intervals during the course of each series of experiments, and femoral bone marrow, lymph nodes and spleen were removed for histologic study. Tissues were fixed in either Bouin's fluid (picric acid–acetic acid–formaldehyde solution) or Maximow's fluid (Zenker solution prepared with formaldehyde solution), dehydrated in alcohols, cleared in xylene, infiltrated and embedded in 56-58 degree paraffin. Sections were stained with Delafield's hematoxylin and with azure II-eosin.

EXPERIMENTAL OBSERVATIONS AND RESULTS

GROUP 1.—In this small group of 3 animals only 2 cc. of benzene–olive oil mixture per kilogram was injected daily over a period of forty days. As the effects on the blood and blood-forming mechanism were inconclusive, they will be considered only briefly.

Peripheral Blood.—In each rat there occurred during the first two days a slight rise in total number of leukocytes per cubic millimeter of 500 cells, followed by a rapid fall to about one fourth of the initial value. Several fluctuations followed in cycles of from four to seven days, the high points in each cycle gradually decreasing and the low points gradually increasing, the average of high and low points in the last cycle before the animals were killed being, respectively, 9,500 and 5,500 cells.

Neutrophils, which averaged 30 per cent at the beginning of the experiment, went through a fluctuating rise during the course of the experiment, averaging 70 per cent after twenty days and 50 per cent after forty days. Staff forms gradually increased from 0 to 20 per cent and myelocytes from 0 to 5 per cent. The absolute number of neutrophils after forty days' treatment was three fifths of the initial number.

The lymphocyte percentage, conversely, gradually decreasel from an initial 70 to 40 after forty days, the absolute number falling to about one third of its initial value. During this period the percentage of larger lymphocytes increased from 0 to 6. On the seventh day and thereafter lymphocytes showing evidences of toxic irritation in the form of deep blue cytoplasm with azure granules and lighter staining nuclei with coarse chromatin aggregates were present, averaging 10 per cent of the total number of lymphocytes after forty days.

Several fluctuations in the number of erythrocytes were encountered in this group, the count returning to the initial value, however, after fourteen days. Hemoglobin values fluctuated with the total erythrocyte count, the cells being normochromic. Reticulocyte percentages increased to values of from 5 to 7 during periods of rising count.

Hemopoietic Organs.—In general, the marrow appeared to be of normal consistency after forty days' treatment, the only change noted being a shift to younger forms in neutrophilic granuloblasts.

Little change was found in either the spleen or the lymph nodes except a decrease in size of the lymphatic nodules. Individual lymphocytes gave evidences of response to toxic irritation, as indicated by nuclear shrinkage and increased cytoplasm. Also, in some cells the nuclear chromatin was collected into large clumps, and the cytoplasm was stained a deep muddy blue. No excessive deposition of hemoglobin pigments was encountered.

GROUP 2.—In this group of 4 animals daily injections of 3 cc. per kilogram of benzene–olive oil mixture were given over a period of from three to sixty days. A gradual loss of weight was noted, amounting to about one third of the original weight after thirty days. Coincidentally, external symptoms, including incoordination, weakness, inattention and evidences of slight hemorrhage, became evident.

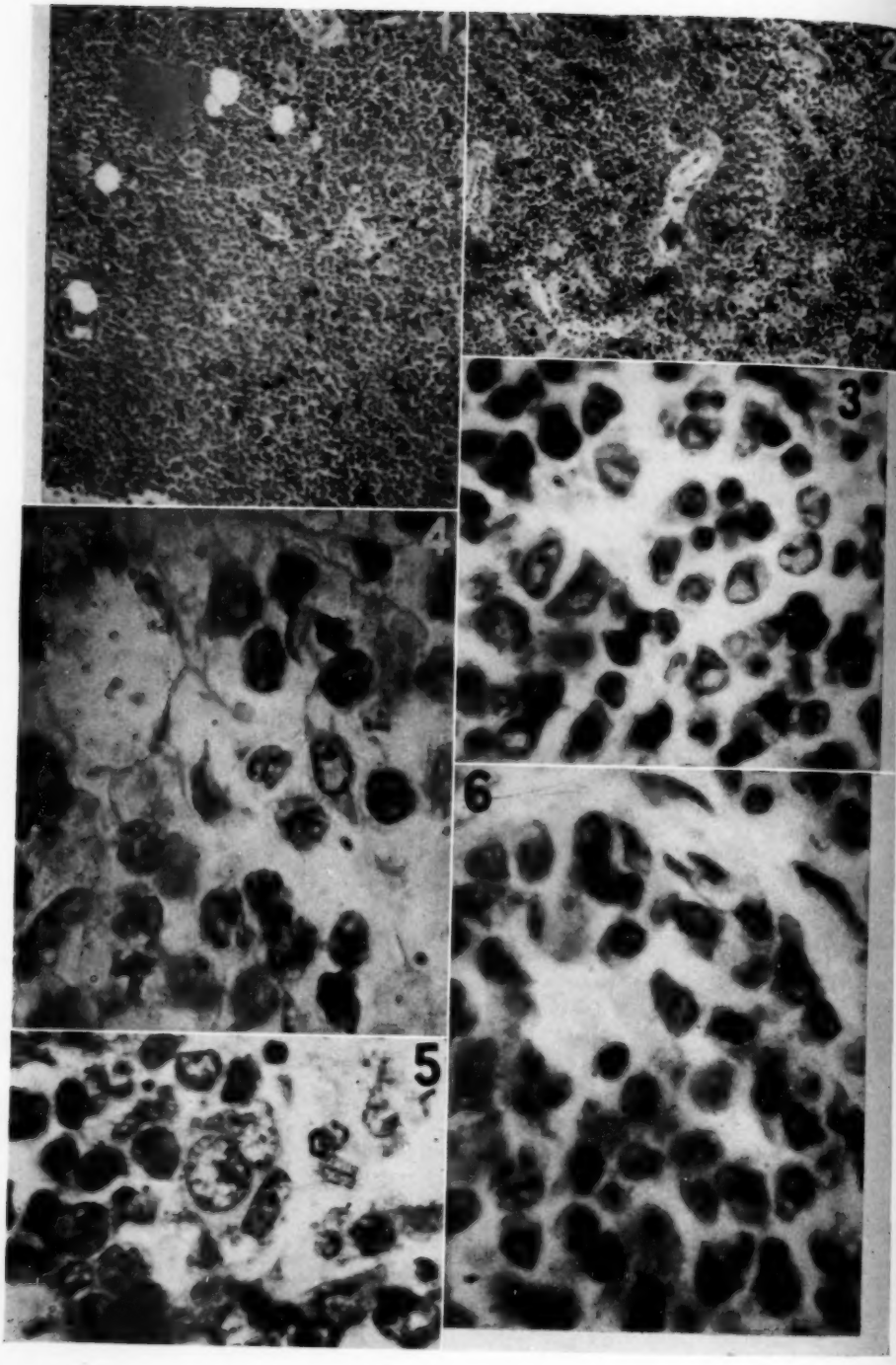
Peripheral Blood.—Cyclic fluctuations in the total number of leukocytes, similar to those noted in the first group, were encountered in this series. A primary rise of from 1,000 to 2,000 cells was followed by a sharp decrease of from 7,000 to 8,000 cells, at which time (ten days) 1 animal died. In another animal the count continued to fall gradually with slight variations, reaching a value of 500 cells per cubic millimeter after twenty-one days. The 2 remaining animals survived this initial decline in total count, and during the following few days the leukocyte count increased to about three fourths of its original value, where it tended to remain (9,000 to 10,000 cells per cubic millimeter).

The percentages of neutrophils and lymphocytes (30 and 70, respectively) remained practically unchanged with one exception, the absolute numbers varying with fluctuations in the total count. The absolute number of neutrophils decreased from about 4,000 to 3,000 cells per cubic millimeter and lymphocytes from about 9,500 to 7,000 cells per cubic millimeter. However, staff forms of neutrophils rarely seen in the normal smears, reached 10 per cent of the total, and myelocytes were occasionally encountered in later stages. Similarly so-called irritation lymphocytes, not seen in normal smears, reached 10 per cent of the total number of leukocytes, and quite large lymphocytes were occasionally encountered.

In the exceptional rat which died after ten days (A-3) there occurred a relative increase in neutrophils from 20 to 60 per cent, an increase in staff forms from 1 to 20 per cent and in later stages an increase in myelocytes to 4 per cent. Marked changes attributed to the toxic irritation of the benzene were present in segmented neutrophils as deeply stained, muddy cytoplasm and toxic granules. There was a 16 per cent increase in the absolute number of neutrophils, with a 35 per cent decrease in the absolute number of lymphocytes.

With the exception of 1 animal (A-6), which presented marked leukopenia after twenty-one days' treatment, practically no effects on erythrocytes or on erythropoiesis were noted in this group. In the rat with severe leukopenia there was during the same period a corresponding decrease in erythrocytes from 8,000,000 to 1,500,000 per cubic millimeter with no increase in reticulocytes.

Hemopoietic Organs.—(*a*) Marrow: The femoral marrow recovered from the rat that died on the tenth day (A-3) was definitely hyperplastic, and no fat cells were present. Foci of polychromatophilic and normoblastic erythroblasts were more numerous than in the normal marrow (figs. 2 and 3). There were slight evidences of granuloblastic hyperplasia with a greater number of mature forms. After thirty-three days similar evidences of stimulation were seen. Some myelocytes were seen, however, with muddy, darkly stained, sometimes vacuolated cytoplasm and nuclei with chromatin collected in masses along the nuclear membrane, evidently in the process of disintegration. Occasional vacuolated macrophages containing phagocytosed cell fragments were also encountered.



Figures 1 to 6

(See legend on opposite page)

Conversely, the marrow of A-6 which died on the twenty-first day with very low leukocyte and erythrocyte counts, was markedly hypoplastic. Myelocytic hypoplasia was most marked, only very early myelocytes and mature forms being identified. Many myelocytes were seen in stages of degeneration. Reticular cells were prominent (fig. 4). Erythroblastic foci and megakaryocytes were also markedly reduced.

(*b*) Spleen and Lymph Nodes: A-3, which died on the tenth day, showed the nodular lymphatic tissue of the spleen and nodes greatly decreased as compared with the controls. In both organs there was an evident increase in trabecular fibrous tissue. Increased numbers of macrophages and swollen reticular cells lining the sinuses were particularly evident in the lymph nodes. Degenerative changes were seen in many lymphocytes, expressed in muddy, deep-staining cytoplasm and either pale, achromatic nuclei or nuclei with large chromatin aggregates inside the nuclear membrane (figs. 10 and 11). After thirty-three days the histologic appearance of these organs was little changed except for increased fibrosis in the capsules and trabeculae and further increase in the number of macrophages. In A-6, which died on the twenty-first day, the lymphatic tissue in both organs was tremendously reduced, there being no nodular aggregates. Fibrous overgrowth was much more marked than in the preceding instances, and degenerative features, as just described, were found in practically all lymphocytes, and both nodal and splenic sinuses were packed with macrophages.

GROUP 3.—This group consisted of 16 animals subjected to daily injections of 4 cc. per kilogram of benzene–olive oil mixture. Symptoms of distress and loss of weight were more severe than in the preceding group. Animals died or were killed in such order that a series of tissues representing from three to sixty days of treatment was studied.

EXPLANATION OF FIGURES 1 TO 6

Fig. 1.—Marrow from the femoral shaft of a normal animal, showing the cellular consistency and relative amount of erythropoietic tissue. × 110.

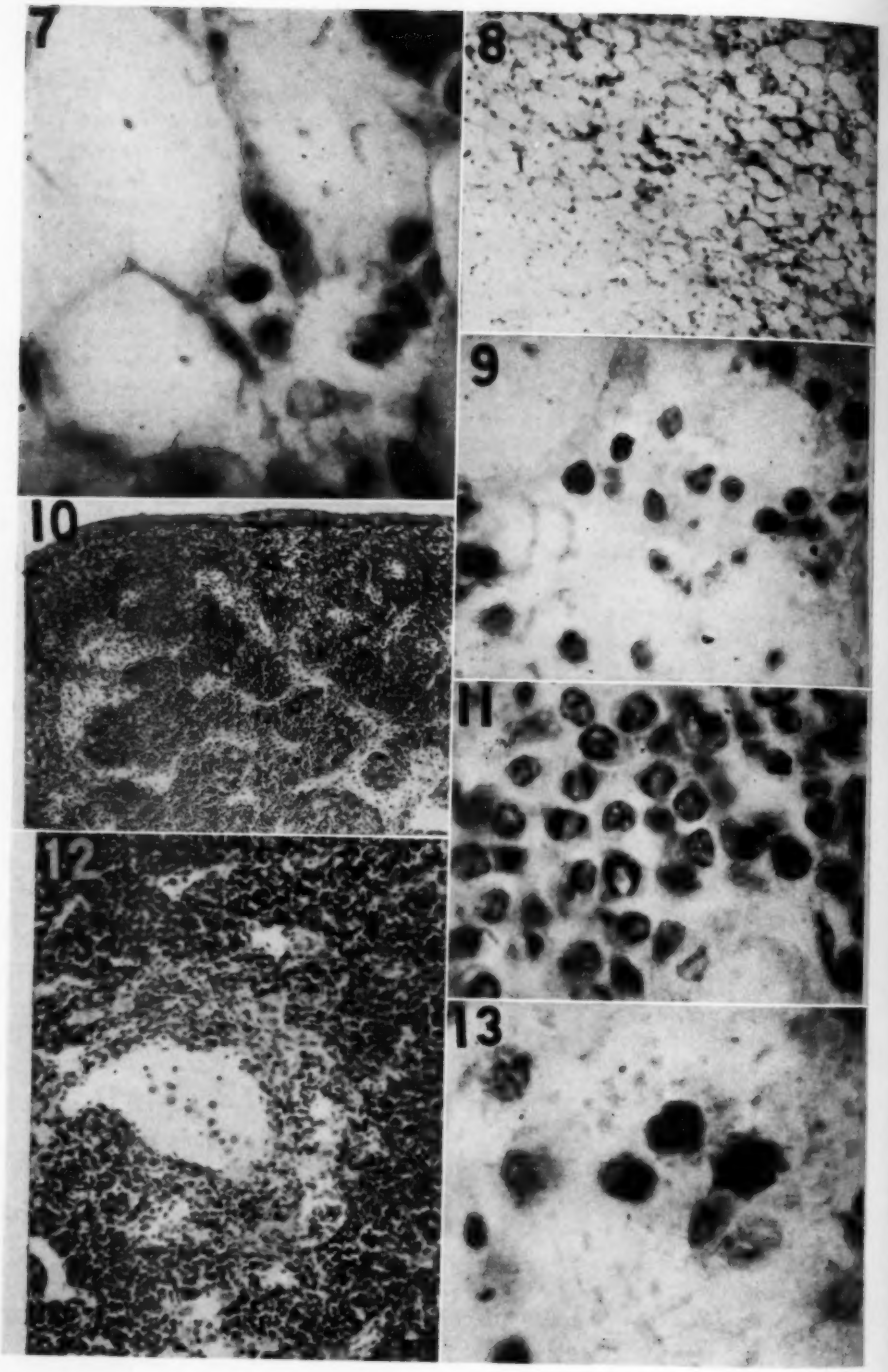
Fig. 2.—Femoral marrow from an animal subjected to 3 cc. per kilogram of benzene–olive oil daily for ten days. Erythroblastic hyperplasia is definitely shown. No fat cells are to be found. × 110.

Fig. 3.—Same marrow as in figure 2 under higher magnification. Both erythroblastic and granuloblastic stimulation are indicated here. The distribution of nuclear chromatin and the deep cytoplasmic basophilia in some cells gives evidence of toxic irritation. × 980.

Fig. 4.—Higher magnification of the hypoplastic marrow from an animal that died after twenty-one days' treatment. Reticular cells with macrophagic tendencies, very early myelocytes and mature granulocytes only are encountered. Evidences of nuclear degeneration are prominent. × 980.

Fig. 5.—Femoral marrow from an animal subjected to 4 cc. per kilogram of the mixture daily for six days. Large macrophages are seen, some with vacuolated cytoplasm. Other degenerative features, as indicated earlier, are obvious in the myelocytic forms. × 980.

Fig. 6.—Femoral marrow from an animal after thirty-five daily injections. Most of the granuloblasts present exhibit characteristic clumping of chromatin and deep cytoplasmic basophilia. No early erythroblasts are seen. Reticular cells appear swollen. × 980.



Figures 7 to 13

(See legend on opposite page)

Peripheral Blood.—In all cases in this group a sharp decrease in the total number of leukocytes occurred in from three to eight days from initial values of 13,000-29,000 cells to 1,500-1,700 cells per cubic millimeter. Cyclic fluctuations followed, as in other groups, completed in eight to ten days, during which the count increased by 3,000 to 5,000 cells, but the mean count gradually lowered, reaching 500 to 1,500 cells per cubic millimeter in those animals surviving after two months.

The percentage of neutrophils decreased sharply during the early phase, from 30 to practically 0. Following a short period of fluctuation, the percentage rose rapidly to between 60 and 70, around which point it fluctuated during the remainder of the experiment. A marked left shift appeared early, staff forms of neutrophils rising to 40 per cent of the total number of leukocytes during the first ten days, but declining gradually to arrive at a value of 2 to 3 per cent of the number of leukocytes at sixty days. Juvenile forms fluctuated correspondingly, reaching a high point of 10 per cent. The absolute number of neutrophils fluctuated with the total leukocyte count, reaching a value of from one third to one half of the initial number after sixty days.

The percentage of lymphocytes rose during the early phase of rapidly decreasing total leukocyte count, from 70 to 90. After several days of fluctuation between these values, it decreased rapidly in four to six days to between 10 and 30. As injections continued, there was a gradual increase in irritation (degenerative)

EXPLANATION OF FIGURES 7 TO 13

Fig. 7.—Detail of femoral marrow as found after forty-five daily injections. The marked aplasia of the marrow with fat replacement is obvious. Most of the nuclei seen are densely pyknotic. Much cellular debris is present. A few large degenerate-appearing reticular cells are to be seen. × 980.

Fig. 8.—General appearance of femoral marrow after sixty daily injections of 4 cc. of the mixture. The marrow is almost completely aplastic. × 110.

Fig. 9.—Higher magnification of the marrow shown in figure 8. Only a few widely scattered cells in varying stages of degeneration are to be found. Most nuclei are pyknotic; a few show clumping of chromatin at the nuclear membrane. Much cellular debris is shown. × 980.

Fig. 10.—Submental lymphatic node from an animal given daily injections of 3 cc. of the mixture per kilogram for ten days (see fig. 2). The sinuses are very prominent and contain numerous macrophages. The lymphatic tissue is correspondingly reduced. Nodular centers are not apparent. × 61.

Fig. 11.—Higher magnification of the lymphatic tissue shown in figure 10. Marked clumping of chromatin and deeply basophilic toxic cytoplasm are well shown. Sharply defined nonstaining areas are seen in the cytoplasm of some lymphocytes. × 980.

Fig. 12.—Portion of a lymphatic node from an animal after thirty-five daily injections of 4 cc. of the mixture. Marked increase in macrophages in and bordering the sinuses is well shown. × 110.

Fig. 13.—Detail of a node after sixty days of injection. Most of the parenchymal cells have disintegrated, and cellular debris is prominent. The few cells remaining show either extreme pyknosis or clumping of chromatin and deeply basophilic cytoplasm. Some of these degenerating cells are of macrophage proportions. This extreme aplasia was accompanied by fibrous overgrowth. × 980.

lymphocytes with very deep blue cytoplasm and distorted nuclei; in terminal stages practically all lymphocytes were of this type. The absolute number of lymphocytes varied with the total leukocyte count but was tremendously reduced during the second month, to reach a final value of only 2 to 3 per cent of the number of leukocytes.

A gradual but eventually severe anemia developed in those rats subjected to prolonged treatment. The erythrocyte counts dropped from an initial 5,000,000-7,000,000 cells to 1,500,000-2,500,000 cells per cubic millimeter for those rats surviving after sixty days. The hemoglobin percentage varied with the total count of erythrocytes, and the reticulocyte percentage never exceeded the normal.

Hemopoietic Organs.—(*a*) Marrow: After three or four days' treatment the sections revealed a suggestion of granuloblastic hyperplasia only, the tissue being otherwise normal. After six days, macrophages with pigment inclusions were found, and granuloblastic hypoplasia was evident. Numerous degenerating myelocytes possessed nuclei with chromatin aggregates along the nuclear membrane, sometimes vacuoles, and flaky cytoplasm with ragged borders (fig. 5). Mature neutrophils were more numerous than in normal marrow. After sixteen days myeloid hypoplasia was more apparent, with a further shift toward mature neutrophils. Degenerative features in myelocytes, just described, were much more widespread. In many mature myelocytes specific granules appeared only in the nuclear *Hof*, and many naked nuclei were encountered. Pigment-laden macrophages were more numerous, and some hemoblasts with the characteristic degenerative features were present. After this stage (twenty-four days) no typical myelocytes were seen, but many mononuclear cells were present, possessing pale, vacuolated nuclei and deeply basophilic cytoplasm. Deeply pyknotic nuclei were encountered in some myelocytes. Macrophages with a noticeably increased amount of included pigment were prominent. After thirty-five days practically all macrophages contained hemosiderin, and many of them exhibited, in response to the toxicity of benzene, the characteristic degenerative features—vacuolated, flaky, deep blue cytoplasm and clumping of nuclear chromatin. Erythropoietic activity was noticeably diminished (fig. 6). After forty-five days no myelopoietic activity was apparent, but erythropoiesis was not further depressed, groups of normoblasts being occasionally encountered. Small necrotic areas were infrequently found. Toxic, degenerating macrophages were further increased with greater amounts of phagocytosed pigment. Most of the hemoblasts contained curious defined areas of cytoplasmic basophilia separated sharply from unstained areas. Such cells possessed distorted nuclei, as described earlier. Megakaryocytes were absent (fig. 7). After sixty days the marrow was almost completely aplastic (fig. 8). A few mature granulocytes with typical degenerative features and scattered normoblasts were found (fig. 9). Blood pigment was prominent, both within macrophages and free in the tissues.

(*b*) Lymph Nodes and Spleen: As in the previous group, changes in the lymphatic tissue of these organs appeared early. After four to six days there was a demonstrable reduction in lymphatic tissue, and many lymphocytes possessed an increased amount of cytoplasm. Sinuses were filled with cells identified as intermediate between lymphocytes and monocytes. Fragmentation and pyknosis of nuclei were apparent throughout. Many reticular cells exhibited the characteristic response to the toxic irritation of benzene—increase in amount of basophilic cytoplasm and clumping of chromatin. All stages in the rounding up of reticular cells to form macrophages were found, particularly in the spleen. The cells of reaction centers of splenic nodules were obviously undergoing typical degeneration. After sixteen days nodal sinuses were much increased in size and filled with foamy,

vacuolated macrophages. Lymphocytes were much reduced in number, and all of them exhibited the characteristic degenerative features. Splenic nodules were very small, and no cortical nodules were seen in the nodes. A definite increase in connective tissue of the capsules and trabeculae of both organs was noted. After twenty-four days the features described were much more marked. Huge vacuolated macrophages (fig. 12) were very prominent in both organs, and reduction of lymphatic tissue, fibrosis, and disintegration of cells were much advanced over the preceding stage. After thirty-five and forty-five days lymphocytic hypoplasia and fibrosis were still more advanced and after sixty days lymphocytes had practically disappeared from both the nodes and spleen, and fibrosis was very marked. Huge, degenerated, vacuolated macrophages were still prominent, and cells recognized as plasma cells were present. The few scattered lymphocytes all showed the typical degenerative reaction to benzene as described. There was abundant cellular debris.

COMMENT

Study of the results just described justifies the conclusion that benzene had a primary stimulative effect on the neutrophilic granulopoietic system. In groups 1 and 2, in which lower doses were used, the total leukocyte count increased during the first few days of injection. This was followed by a sharp decrease in the total count, indicating a peripheral destructive effect on circulating leukocytes. This peripheral destruction was apparently concomitant with a continued stimulative action on the granulopoietic tissue of the marrow, as was shown by the granuloblastic hyperplasia observed in the marrow of the rat of the second group (fig. 3) which died on the tenth day of injections and in the marrow from the rat of the third group after four days. When lower doses were given (3 cc.) granuloblastic hyperplasia was still encountered in 2 cases after thirty-three days. A similar increase in peripheral count and primary myelocytic hyperplasia in rabbits following injection of benzene have been reported by Selling [1] but not by other investigators. When larger doses were given (group 3), the primary stimulation of marrow noted in the other groups was overshadowed by the destructive action on the circulating cells, no primary increase in the total count being found and the percentage of neutrophils being reduced to almost 0 in the first few days, even in the presence of slight myelocytic hyperplasia in the marrow. A response of the hyperactive marrow to this rapid depletion of circulatory neutrophils is seen in a later increase in the blood of the percentage of staff, juvenile and myelocytic forms. Further evidence of the combined stimulative and destructive action of benzene on cells of the leukocytic series is found in the cyclic variations in total leukocyte count encountered in most animals of all three experimental groups. Similar fluctuations in total leukocyte count as a result of introduction of benzene have been reported by Weiskotten and his associates.[4]

4. (*a*) Weiskotten, H. G.: Am. J. Path. **6**:183, 1930. (*b*) Weiskotten, H. G.; Schwartz, S. C., and Steensland, H. S.: J. M. Research **33**:127, 1915; **35**:63, 1916. (*c*) Weiskotten, H. G., and Steensland, H. S.: ibid. **39**:485, 1919.

In the first group the concentration of oxidation products of benzene (which, according to Kracke, is responsible for degenerative changes) apparently was not sufficient to produce any marked changes during the experimental period. A possible slight increase in percentage of promyelocytes and myelocytes in the marrow was reflected in the blood by an increase in staff forms and myelocytes. These appearances and the gradual decrease in absolute numbers of neutrophils could be largely explained on the basis of increased strain due to peripheral destruction.

In the second group some degenerative effects were encountered in the marrow. In a rat in which total leukocytes markedly decreased during a twenty-one day period, marked granuloblastic hypoplasia was noted in the marrow, and degenerative signs were found in all myelocytic forms. The myelocytic hypoplasia was accompanied by some degenerative features. After ten days of treatment there were no evidences of degeneration. Presumably, then, in this group most of the disturbances in neutrophil count were due to destruction in the peripheral blood and stimulation of the marrow, staff forms and myelocytes being present in increasing percentage in the blood.

In the third group degenerative features were seen in myelocytic cells in the marrow examined after only six days of treatment. Hypoplasia and degenerative features in cells of the myeloid series were very evident after eleven days, and after twenty-four days no typical myelocytes were present in the marrow. These signs became more and more pronounced; granuloblastic tissue was absent and necrotic areas were found after forty-five days and practically complete aplasia of femoral marrow after sixty days. In view of these findings it is not surprising that the absolute number of neutrophils in the circulation should decrease during sixty days to from one third to one half of its original value.

Examination of the spleens and lymphatic nodes showed that benzene or its oxidation products had a definitely greater toxic effect on lymphatic tissue than on the myeloid tissue. In the first group, in which little demonstrable change could be seen in the marrow, there was definite hypoplasia of lymphatic tissue, and irritation forms of lymphocytes were scattered throughout. This explains the decrease in percentage of lymphocytes from 70 to 40, the increase in percentage of larger lymphocytes and the appearance and gradual increase in the number of irritation lymphocytes in the peripheral blood.

This greater sensitivity of lymphocytes was more noticeable in the second group, in which hypoplasia, fibrosis and degenerative signs were found in lymphatic tissue of the spleen and nodes after ten days' treatment. The same toxic features were found after thirty-three days, only to a more marked degree. In the animal which succumbed after twenty-one days there was generalized reduction of lymphatic tissue, and practically all lymphocytes showed degenerative changes. In all cases

there were evidently a great many lymphocytes and reticular cells undergoing transformation into macrophages. These features in the lymphatic tissue were reflected in the blood by the absolute decrease in lymphocytes and the increasing numbers of large and irritation lymphocytes in later stages.

As might be expected, such changes as were found in the preceding groups appeared earlier in the individuals of the third group, which received higher dosages. After only four days practically all tissue lymphocytes showed an increased amount of cytoplasm, and nodal and splenic sinuses were crowded with cells classified as intermediate between lymphocytes and monocytes. After sixteen days the sinuses were very prominent and filled with huge, very vacuolated, degenerate-appearing macrophages. No nodular lymphatic tissue was present, and the scattered lymphocytes all gave evidence of toxic irritation. Later (twenty-four days) lymphocytic degeneration and fragmentation became very marked and increase of fibrous tissue prominent. Lymphatic tissue became progressively more aplastic, and signs of degeneration, fibrosis, and production of macrophages greater at thirty-five, forty-five and sixty days. Again these marked degenerative features can be correlated with the great decrease in lymphocytes to 2 to 3 per cent of the original value in the blood, the gradual increase in irritation forms and the final degenerative appearances in practically all blood lymphocytes.

From both blood and marrow studies it was apparent that circulating erythrocytes and erythropoietic tissue were more resistant to the toxic effects of benzene. With a low dose (group 1) some fluctuation in the total number of erythrocytes was found, doubtless due to varying degrees of stimulation and depression of erythropoietic centers. A balance between these factors was eventually established, and the number of cells returned to normal in later stages of the experiment. Consequently, the marrow appeared normal as regards erythropoietic activity. With an increased dose (group 2), in only 1 rat (twenty-one days) was any significant change in erythrocyte count encountered. In this animal progressive and severe anemia occurred with no evidence of regeneration. The marrow in this rat exhibited marked hypoplasia of erythroblastic as well as of myeloid tissue.

When a still heavier dose of benzene was given (group 3), slowly progressive and ultimately severe anemia developed, with no signs of regenerative activity appearing, such as the presence of normoblasts or an increase in reticulocytes. From examination of the marrow in this series it was apparent that the benzene did not cause any degeneration of erythroblastic tissue already present, but rather that it in some way interfered with mitotic activity and the processes of maturation, as erythroblastic hypoplasia appeared gradually, first pronounced after thirty-five days of treatment. The divergence of reticular cells into the

production of numerous toxic-appearing macrophages and the direct degeneration of stem cells and their frequent transformation into plasma cells were undoubtedly factors in the development of anemia as well as in that of leukopenia. Increased destruction of erythrocytes, either because of increased fragility or because of the activity of the increasing number of macrophages, was indicated by the gradual increase in the number of macrophages containing hemosiderin pigment. This feature was first noted in the marrow after eleven days, gradually increased and was very marked after sixty days' treatment.

These erythrocytic changes encountered in the experiments we report were of much greater magnitude than those reported by previous investigators. Selling,[1] in 1911, had produced aplastic marrows in guinea pigs, and others (Hurwitz and Drinker;[5] Weiskotten, Schwartz and Steensland [4b]) have agreed that aplasia of the marrow can be produced with massive doses.

The nature of the degenerative changes in response to the toxic irritation of benzene encountered in lymphocytes, myelocytes and leukocytes received considerable study. The first evidences of change occurring in the lymphatic tissue were an increase in the amount of cytoplasm and an increase in its basophilia. As the toxicity increased, the intensely basophilic cytoplasm appeared muddy or flaky, and vacuoles of various sizes appeared. Simultaneously, the nuclei of such cells became somewhat shrunken and either were pale and relatively achromatic or had quite heavy chromatin aggregates just inside the nuclear membrane. As the dose increased, more and more lymphocytes were seen in stages of disintegration, and much cellular debris was found. In addition to these appearances, after the administration of heavy doses was started, cells intermediate in nature between lymphocytes and monocytes filled many sinuses of the lymph nodes, and reticular cells appeared to be in the process of rounding up. In later stages of the experiment most of such cells had assumed the proportion of macrophages, with shrunken nuclei and large amounts of foamy cytoplasm filled with vacuoles. In many cases lymphocytes had become changed into cells resembling plasma cells.

In the myeloid tissue the first degenerative signs appeared in neutrophilic myelocytes. After thirty-three days with lower doses and after six days with higher doses, numerous myelocytes were found with degenerative features similar to those described. Most of these possessed very few specific granules, regardless of the nuclear configuration. These granules often were inside small vacuoles. As the toxicity increased (sixteen days, group 3), some of the cells of this nature had disintegrated, and changes were encountered in the hemoblasts present, exactly as described in

5. Hurwitz, S. H., and Drinker, C. K.: J. Exper. Med. **21**:421, 1915.

lymphocytes. In still later stages (twenty-four days) no normal myelocytes appeared, and the mononuclear cells present exhibited characteristic degenerative features. The transformation of reticular cells into huge, foamy degenerate-appearing macrophages, most of which had phagocytosed hemosiderin, was a marked feature in later stages of the experiments of the third group.

SUMMARY

Repeated daily injections of a benzene–olive oil mixture in rats produced a primary stimulation of the neutrophilic granulopoietic system, shown by increase in blood neutrophils and hyperplasia of myelocytic marrow. This was followed by rapid destruction of circulating leukocytes, with partial marrow compensation being indicated by a marked left shift in blood neutrophils and cyclic fluctuations in count. Degenerative features appeared next in myelocytes, varying in degree with the degree of benzene intoxication. With heavy doses, no normal myelocytes were found after twenty-four days, and there was complete marrow aplasia after sixty days.

Lymphatic tissue showed much greater sensitivity to benzene in that degenerative features appeared earlier and progressed with greater rapidity than in the marrow. Lymphatic hypoplasia, fibrosis, lymphocytic degeneration and transformation of lymphocytes, monocytes and reticular cells into macrophages progressed rapidly until with heavy doses practically all lymphatic tissue had disappeared from the spleen and lymph nodes. Coincidentally, blood lymphocytes became reduced to between 2 and 3 per cent of their original value, none of them of normal appearance.

Erythropoietic tissue and circulating erythrocytes were more resistant to the toxic effects of benzene. With heavier doses, a slowly progressive, ultimately severe hemolytic anemia developed. Arrest of maturation and direct degeneration of stem cells or their transformation into macrophages finally resulted in marked erythroblastic hypoplasia.

Characteristic degenerative changes in lymphocytes, monocytes, myelocytes, reticular cells and hemoblasts consisted in an increase in the amount and basophilia of the cytoplasm, which then became muddy, flaky and vacuolated. Simultaneously, the nuclei became either pale and achromatic or the chromatin became clumped into a few large aggregates at the nuclear membrane. Under these conditions specific granules were not differentiated in myelocytes. Cellular disintegration was the end result. Under severe intoxication the increasing number of macrophages became very large, with foamy, vacuolated cytoplasm and very shrunken nuclei, preliminary to their complete dissolution.

EFFECT OF CONSTRICTION OF THE RENAL ARTERIES IN PREGNANCY AND IN CERTAIN ENDOCRINE STATES OF RABBITS

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The method for the production of hypertension in dogs by constriction of the renal arteries was described by Goldblatt and collaborators[1] and has since been used successfully by many workers. This method or some modification of it has been used to accomplish hypertension in the monkey,[2] rat[3] and rabbit,[4] and the important pathologic changes have been reported.[5]

We have utilized a comparable method of constricting the renal arteries to produce renal ischemia and renal injury with resulting hypertension in pregnant dogs and rabbits. Our preliminary observations[6] describe increased susceptibility and significant hepatic lesions occurring in pregnant dogs and rabbits subjected to such constriction of the renal arteries. These observations suggest the similarity of the physiologic and pathologic processes occurring in these experiments to those associated with at least some of the human toxemias of pregnancy. This suggested to us that the method of investigation described offers a technic for the production of "test animals" in which the factors of eclampsia may be studied; pregnancy, renal injury with or without insufficiency, acute and chronic hypertension, diet and other factors may be varied almost at will. The many debatable and controversial theories in regard to multiple pregnancies, vascular changes, renal infection, blood protein levels, products of tissue degeneration and similar possible etiologic factors may be tested in the hypertensive pregnant experimental

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From the Department of Pathology, Duke University Medical School.

1. Goldblatt, H.; Lynch, J.; Hanzal, R. F., and Summerville, W. W.: J. Exper. Med. **59**:347, 1934.

2. Goldblatt, H.: J. Exper. Med. **65**:671, 1937.

3. Rytand, D. A.: Proc. Soc. Exper. Biol. & Med. **38**:10, 1938.

4. Wilson, C., and Pickering, G. W.: Clin. Sc. **3**:343, 1938.

5. (*a*) Child, C. G.: J. Exper. Med. **67**:521, 1938. (*b*) Goldblatt, H.: Ann. Int. Med. **11**:69, 1937; (*c*) J. Exper. Med. **67**:809, 1938. Goldblatt and others.[1] Goldbatt.[2] Rytand.[3] Wilson and Pickering.[4]

6. Dill, L. V., and Erickson, C. C.: Proc. Soc. Exper. Biol. & Med. **39**: 362, 1938.

animal and viewed in a picture comparable to the pregnancy toxemia of man.

It has been shown by Greene [7] that the various phases of the reproductive cycle are significant in the occurrence of the toxemia of pregnancy as it occurs spontaneously in the rabbit. Similarly, we have studied such variations in the rabbit, for this animal is a most suitable "test animal" in which the various endocrine states are the variable.

This report records our further investigations of the response of pregnant rabbits with constriction of the renal arteries as contrasted with the reactions of control groups of: normal female rabbits without pregnancy or pseudopregnancy; spayed and pseudopregnant rabbits with constriction of the renal arteries; pregnant rabbits and normal female rabbits with bilateral nephrectomy.

MATERIAL AND METHODS

Experiment 1.—Twenty-two mature female rabbits, weighing approximately 1,800 to 2,500 Gm., were operated on at the beginning of the last trimester of pregnancy, and 8 young female rabbits, weighing approximately 1,300 to 1,800 Gm., were operated on from the seventeenth to twentieth day of pregnancy (see table, experiment 1, groups A, B and C).

All animals were kept in individual cages on a stock diet of oats, alfalfa and water. Food was omitted for twenty-four hours preoperatively and for twelve hours postoperatively.

Blood pressure was determined on representative groups twice weekly until constriction of the renal arteries or nephrectomy was performed and then daily for ten days, or until the animals died. On dilatation of the vessels of the ear following local application of xylene, it was found that the auricular pulse was of ample volume to be easily palpated. By applying a small cuff around the base of the ear, after the concave surface had been packed with a firm, smooth bolus of cotton gauze, it was found that indirect blood pressure readings could be made by palpation. Such readings are fairly reliable within physiologic blood pressure ranges when the precautions necessary for any indirect method are observed. The readings by this method have been checked by readings direct from the carotid artery of the rabbit.

Operative procedures were done on animals under ether anesthesia. The kidneys were delivered aseptically through the retroperitoneal approach. Both renal arteries were exposed, and a small loop of no. 26 silver wire was so placed as to produce minimal to moderate constriction of the lumen of the main renal artery. The operative wound was closed in layers with black silk.

When an animal was killed inhaled illuminating gas was used. A complete necropsy was performed immediately after death. Urine found in the bladder at death was examined microscopically and qualitatively for albumin. Duplicate blocks were fixed in Zenker's solution and in solution of formaldehyde U. S. P. diluted 1: 10.

Experiments 2 and 3 (Controls).—In order that the effect of constriction of the renal arteries might be studied during other phases of the reproductive cycle, experiment 2 (see table) included three subgroups: Group 2 A consisted of 10 normal mature female rabbits which had been segregated for forty days to rule

7. Greene, H.: J. Exper. Med. **65**:809, 1937.

out pregnancy and pseudopregnancy. Group 2 B i consisted of 8 animals on which total ovariectomy had been performed at least ten days previously, group 2 B ii of 9 animals on which total ovariectomy had been performed three to five months previously and group 2 B iii of 2 spayed animals in which the renal arteries were ligated. Group 2 C consisted of 11 animals which had been rendered pseudopregnant by an intravenous injection of 5 cc. of Berkefeld-filtered pregnancy urine twelve to fifteen days previously. The control animals were subjected to constriction of the renal arteries in a manner and to a degree that would make them comparable with the pregnant animals.

An experiment (see table, experiment 3) designed to rule out the effect of complete removal of functioning kidney tissue and the part played by any secretion

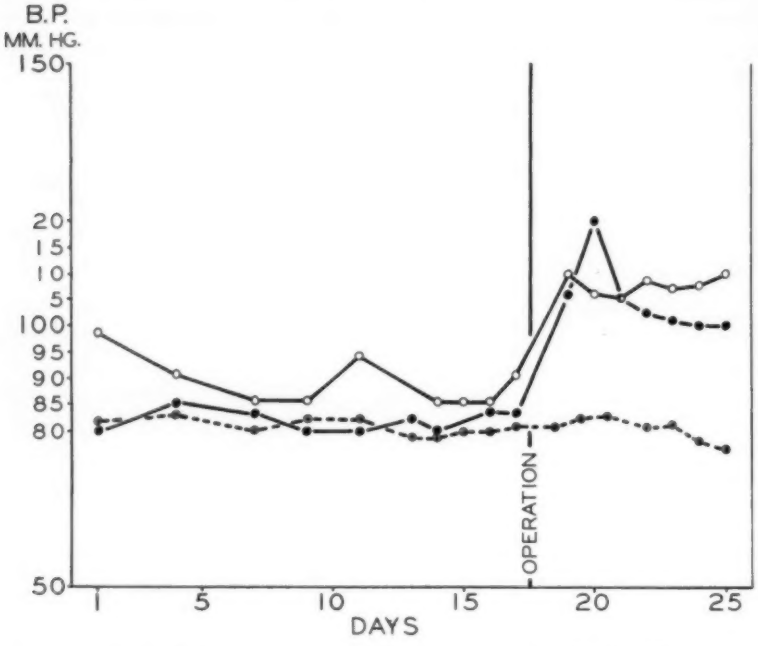

Fig. 1.—Blood pressure in experimental groups of rabbits. Pregnant rabbits with constriction of the renal arteries (solid line with white circles); nonpregnant rabbits with constriction of the renal arteries (solid line with black circles); pregnant and nonpregnant rabbits in which nephrectomy had been done (broken line with black circles).

or degeneration products of the kidney included two subgroups: Group 3 A contained 4 female rabbits in the last trimester of pregnancy; group 3 B, 4 normal mature female rabbits. In both groups the kidneys were exposed by the same technic employed in obtaining constriction of the renal arteries and, following double ligation of the pedicles, were removed.

EXPERIMENTAL OBSERVATIONS

Pregnant Rabbits with Constriction of the Renal Arteries.—Death occurred in 20 of the 30 pregnant animals subjected to renal ischemia,

Experiment	Group	Animals	Degree of Constriction of Main Artery of Each Kidney *	Animals Surviving Given Days †	Heart		Lungs		Kidneys			Liver		
					Dilatation	Necroses and Hemorrhage	Pneumonia	Infarcts and Hemorrhage	Severe	Moderate	Minimal	Severe	Moderate	Minimal
1	A (pregnant 20 days).....	13	+ to +++	2-6 [11] 7-8 **2**	10	4	0	0	2	4	7	6	2	2
	B (pregnant 20 days).....	9	+ to +++	2-5 [9]	3	5	1	2	3	4	2	6	2	1
	C (pregnant 17-20 days).....	(8)	+	Still living										
2	A (nonpregnant).....	10	+ to +++	5 [6], 5 **4**	6	2	3	0	4	3	2	0	2	0
	B i (ovariectomized, recent).	8	+ to +++	5 **8**	**0**	**0**	1	0	2	3	3	0	3	0
	B ii (ovariectomized 3-5 mo.)	9	+ to +++	3 [2], 4 [1] 23 [2], (4)	**0**	**0**	**0**	1	4	3	0	0	0	1
	B iii (ovariectomized).....	2	++++	3-4 [2]	0	0	0	0	2	0	0	0	0	0
	C (pseudopregnant).....	11	+ to +++	5 **9**, (2)	1	1	0	0	4	3	2	0	1	1
3	A (pregnant).....	4	Bilateral nephrectomy	4.5 av. [4]	0	0	0	0	0	0	0	0	0	0
	B (nonpregnant).....	4	Bilateral nephrectomy	3.1 av. [4]	**1**	**0**	1	0	0	0	0	0	0	0
	Total pregnant.....	22 (8)	.....	.....	13	9	1	2	5	8	9	12	4	3
	Total control.....	42 (6)	.....	.....	**8**	**3**	5	1	16	12	7	0	6	2

* Minimal constriction, +, to complete constriction, +++.

† Numbers in parentheses refer to animals still living; numbers in bold face, to animals killed.

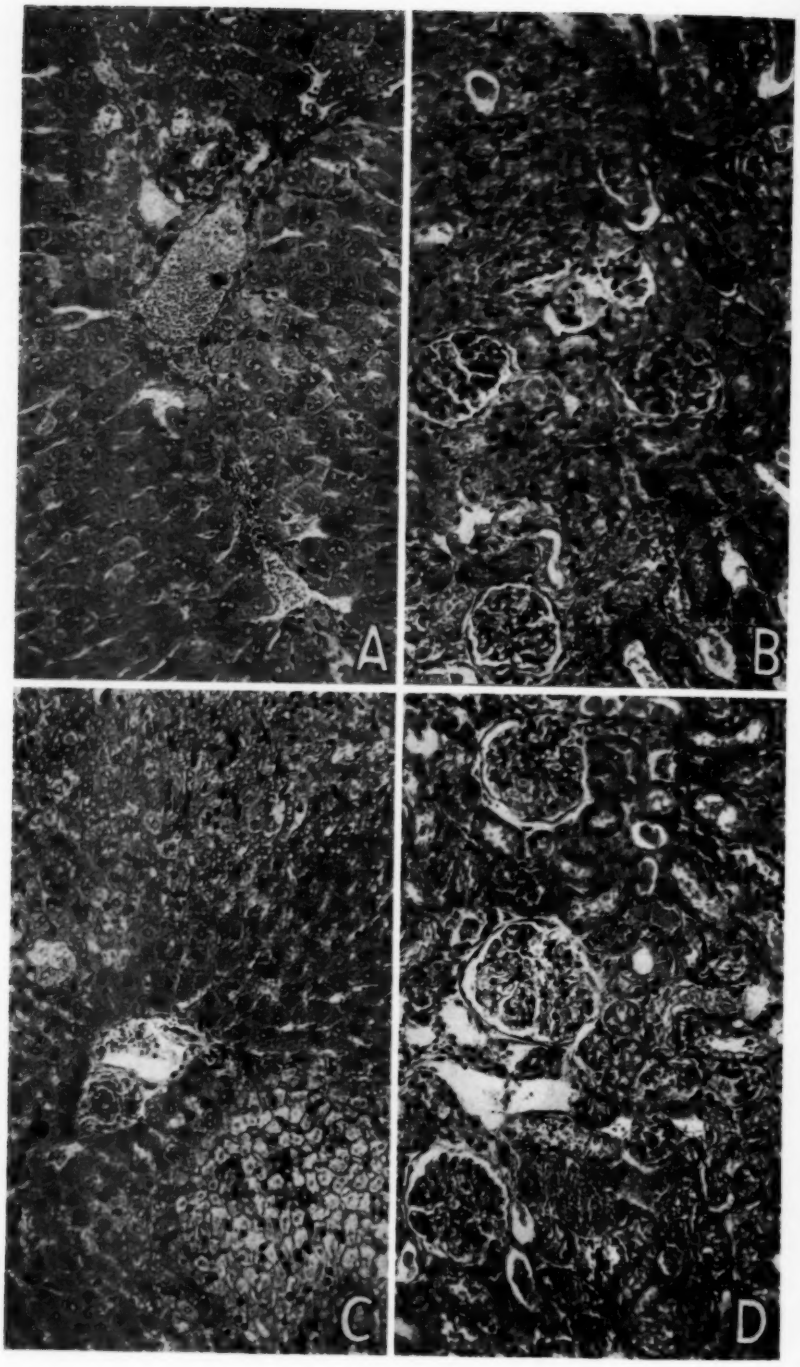

Figure 2

(See legend on opposite page)

within forty-eight to one hundred and fifty hours after operation. These animals demonstrated weakness, anorexia and loss of weight. Four of them were seen in convulsive seizures. One rabbit remained clinically normal for eight days after constriction of the renal arteries and then delivered 6 full term, living fetuses. The animal was killed twelve hours later. Another animal was killed seven days after application of the clamps, while in apparent good health. Eight young female animals, weighing 1,300 to 1,800 Gm., experimental group 1C (table), delivered stillborn young from eight to ten days after operation and have since exhibited no abnormal clinical symptoms. Several animals of this group have been observed in multiple subsequent asymptomatic pregnancies.

In 11 of the 13 animals for which blood pressure readings were recorded, a sustained moderate elevation of systolic blood pressure was noted as measured by a cuff placed on the auricular artery. The average blood pressure elevation is illustrated in figure 1.

Urine found in the bladder at autopsy showed a variable amount of albumin (trace to 4 plus), numerous hyaline and granular casts and occasional red cells. In the urine of animals showing severely injured kidneys an increased number of red blood cells were noted.

Necropsy Observations.—The renal injury as determined by the amount of kidney tissue showing histologic change was severe to moderate in 13 and minimal in 9. Grossly, the minimally injured kidneys showed little more than patchy congestion and slight pallor of the medullary portions with occasional subcapsular hemorrhage and moderate swelling from beneath the cut capsule. In the moderately injured kidneys there were small wedge-shaped areas of discoloration and hemorrhage in the cortex, extending almost to the capsule. Rarely, they were noted in the medullary portion. The more severely injured kidneys demonstrated large wedge-shaped infarcts involving both the cortex and the medulla. Occasionally, the outer two thirds of the cortex appeared completely necrotic, while the medulla showed little evidence of injury.

Microscopically, the kidneys frequently showed subcapsular and focal hemorrhage. The glomeruli were usually not remarkable. Rarely a few red cells were found in Bowman's space. The tubules in the minimally injured kidneys showed swollen epithelium, and one found a rare tubule which had been converted into a homogeneous hyaline mass, which still retained its structure. Other tubules showed little alteration except for occasional hyalinized epithelial cells, an occasional pyknotic nucleus or a moderate amount of hydropic degeneration. In the severely

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EXPLANATION OF FIGURE 2

A, liver of pregnant rabbit 505 after constriction of the renal arteries; hematoxylin–eosin stain; × 150.

B, kidney from rabbit 505; section taken from a subcapsular cortical area; hematoxylin–eosin stain; × 150.

C, liver of pregnant rabbit 504 after constriction of the renal arteries, showing fatty necrosis; hematoxylin–eosin stain; × 150.

D, kidney of rabbit 504; hematoxylin-eosin stain; × 150.

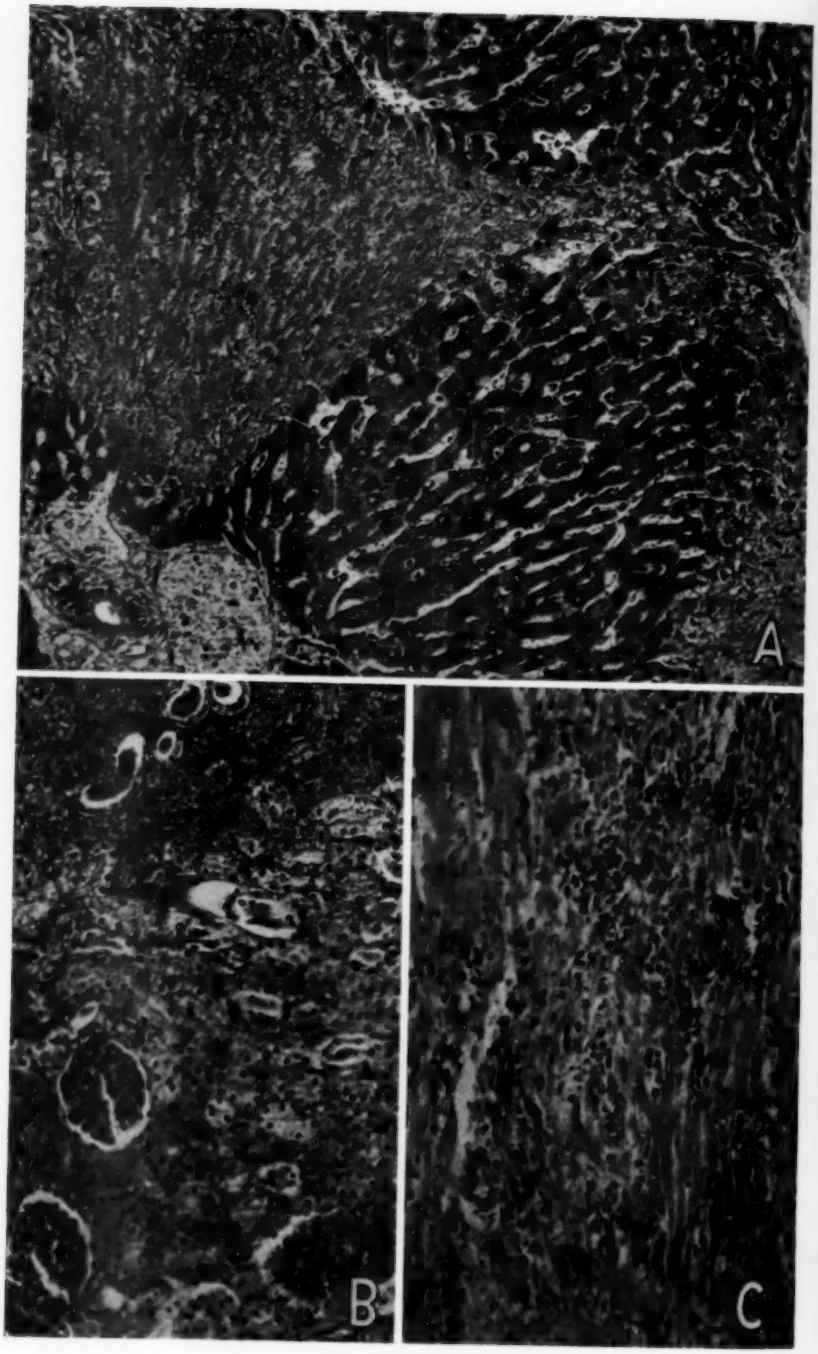

Figure 3

(See legend on opposite page)

injured kidneys one saw fairly normal glomeruli surrounded by severely damaged necrotic tubules. The remaining tubular structures were converted into masses of hyaline material with tubular contours, occasionally containing fragmented and pyknotic nuclei. In some areas necrosis and hyaline change were observed in the glomeruli. Thus the injury varied from those kidneys showing no demonstrable anatomic injury to those which were almost completely infarcted (fig. 2 *B* and *D*; fig. 3 *B*).

In 3 instances kidneys were seen with complete necrosis of the outer two thirds of the cortex but with minimal or no injury to the inner third of the cortex or of the medulla.

The liver grossly was usually moderately enlarged and swollen. The cut surface revealed bulging from beneath the capsule, and the tissue was often noted to be dull and cloudy. Areas of lighter color were frequently apparent around the central areas, giving the liver a mottled, "nutmeg" appearance. Some of the livers were rather greasy and yellow, suggesting fatty change.

Microscopically, 16 of the 22 pregnant animals showed conspicuous hepatic injury of varying degree: widespread central and midzonal injury with necrosis, focal areas of necrosis with cellular infiltration, or extreme generalized fatty changes, most conspicuous in the periportal regions (fig. 2 *A* and *C*; fig. 3 *A*).

In 6 of the animals the predominating lesion was the widespread central and midzonal type of injury; in 6 it was focal necrosis of the liver tissue, and in 3, diffuse fatty infiltration. An earlier stage of the hepatic injury was suggested by cloudy swelling, especially conspicuous around the portal fields, and in some areas this was so marked that the sinusoids were obliterated. The central and midzonal type of necrosis usually began fairly sharply from one-third to one-half the distance between the portal field and central veins, and a thin band of cells showing fatty changes separated the obviously injured tissue from the apparently normal but swollen tissue. As one progressed toward the central vein the fatty change persisted. Interspersed between the atrophic, pale-staining, shadow cells with their pyknotic nuclei could be seen hyalin-like masses, which occasionally contained fragmented nuclei and in some areas hemorrhage.

The focal necroses were most frequently seen as rather sharply circumscribed areas which extended eccentrically from the margin of a central vein, less frequently from the portal areas or were scattered in the midzonal areas without relation to central or portal fields. They were infrequently limited to periportal areas. Practically every liver cell in such necrotic areas was completely fragmented or hyalinized, with nuclear pyknosis, karyorrhexis and karyolysis in the marginal necrotic cells. There was in some instances a polymorphonuclear neutrophil infiltration and in some a macrophage reaction. The sinusoids were usually empty and dilated. Rarely, they contained red cells or macrophages.

EXPLANATION OF FIGURE 3

A, liver of pregnant rabbit 503 after constriction of the renal arteries showing extensive coagulation necrosis; hematoxylin–eosin stain; × 150.

B, kidney of rabbit 503; hematoxylin-eosin stain; × 150.

C, moycardium of pregnant rabbit after constriction of the renal arteries, showing necrosis with cellular infiltration; hematoxylin-eosin stain; × 150.

Fatty infiltration of the liver such as was seen in 2 cases was represented by diffuse vacuolation of practically all of the liver cells. The vacuoles were larger in the periportal areas than in the midzonal or central areas. The sinusoids were not remarkable, and only occasionally did one note definite early degeneration of cells or nuclei.

In 13 of the pregnant animals there appeared to be gross dilatation of the heart, and frequently the myocardium was cloudy. In 3 instances subendocardial and myocardial hemorrhages were noted grossly. Fatty changes in the myocardium were noted in only 2 cases.

Microscopically, the myocardium showed small areas of focal necrosis, located in the subendocardium or in the papillary muscles (fig. 3*C*). These areas of necrosis were characterized by loss of striation, hyalinization and in some places complete disappearance of the myofibrils. Hemorrhage was present but not conspicuous. A considerable amount of cellular reaction was usually present, both polymorphonuclear and mononuclear. A deposit of fine, diffuse calcium was noted in some of the more severely injured fibers.

Grossly, the placenta was usually not remarkable, but in 6 instances it was a hemorrhagic, discolored mass of spongy material.

Microscopically, when infarction was present, these organs showed extensive necrosis. The villi were necrotic, and there were accumulations of fibrin and extensive fragmentation of nuclear structures.

The gastrointestinal tract frequently showed congestion and focal hemorrhages. Definite arteriolar necrosis was not notable.

The pathologic changes of other organs were not significant except for the effects of acute congestion.

Nonpregnant Rabbits with Constriction of the Renal Arteries.—Five of the nonpregnant animals subjected to renal ischemia died within five days (in 4 the renal arteries were severely constricted); the 5 remaining animals were killed at the end of one hundred and twenty hours (renal constriction moderate to severe). The survival time of the group is therefore only suggestively significant. It should be emphasized, however, that all animals with minimal renal injury and many of those with moderate to rather severe damage will survive for an indefinite period.

The symptom complex of the nonpregnant animals in which the renal arteries were severely constricted was not significantly at variance with that of the pregnant group. The animals with minimal to moderate constriction of the arteries had no conspicuous symptoms.

A moderate elevation of systolic pressure as measured by the cuff placed on the auricular artery occurred in all but 3 of the animals (fig. 1).

The analysis of the urine specimen taken from the bladder at postmortem examination showed a moderate amount of albumin, hyaline casts, occasional granular casts and abundant red blood cells when damage was severe.

Necropsy Observations.—The renal injury was severe in 4 of the animals, moderate in 3 and minimal in 3. The type of injury to the kidneys did not differ from that noted in the pregnant animals, although in general more severe destruction had been produced in the nonpregnant animals.

In microscopic sections of 2 of the rabbits, small areas of focal necrosis of the liver were seen, with dead and hyalinized cells, hemorrhage and cellular infiltration. But in no instance was the extensive type of central and midzonal focal necrosis seen in this group as observed in the pregnant animals, although the areas of focal necrosis did show a histologic picture similar to that of the areas of focal necrosis described in the pregnant animal.

Cardiac dilatation was of note grossly in 6 rabbits of this group. In 2 animals multiple areas of focal necrosis were noted microscopically in the myocardium. Lobular pneumonia was seen in 3 animals, all animals with severe renal injury. The other organs showed nothing remarkable other than congestion and occasional focal hemorrhages of the gastrointestinal tract.

Ovariectomized Rabbits with Constriction of the Renal Arteries.—The spayed rabbits in which the kidneys were severely injured did not vary greatly in survival time from the nonpregnant groups. The animals in which moderate and minimal renal ischemia had been produced showed no tendency toward early death and were killed at the end of one hundred and twenty hours.

The blood pressure and urinary findings were similar to those of the pregnant and nonpregnant groups.

Necropsy Observations.—Eight of these rabbits showed severe renal damage, 6 moderate injury and 3 minimal.

Focal necroses of the liver were noted in 3 animals, but no generalized central injury was present in any rabbit of this group.

The histologic changes found in the kidneys and livers of these animals were similar in character to those seen in the previous groups.

Injury to the myocardium was not demonstrated in any animals of this group.

One adrenal showed 2 small areas of necrosis in which considerable numbers of gram-positive cocci could be stained. This was considered to be a direct extension of infection at the operative site, although the autopsy revealed no other evidence of an inflammatory process.

Lobular pneumonia was present in 1 animal of this group. The remainder of the organs disclosed nothing significant.

Pseudopregnant Rabbits with Constriction of the Renal Arteries.—No significant variation in survival time from the other control groups was noted in this group in which constriction of the renal arteries was performed following the production of pseudopregnancy by the injection of pregnancy urine. Animals not dead at one hundred and twenty hours after constriction of the renal arteries were killed.

The animals in which moderate and minimal renal ischemia had been produced were symptom and sign free, while those with severe damage to the kidneys showed the symptoms of uremia.

Necropsy.—In this group renal damage was severe in 4, moderate in 3 and minimal in 2.

In 1 animal focal and central necroses of the liver were seen similar in character to the central injury seen in the 3 rabbits of the pregnant group.

Myocardial injury was present in 1 animal.

Pregnant Rabbits with Bilateral Nephrectomy.—The 4 pregnant animals in which bilateral nephrectomy had been performed died within eighty-four hours, the average survival time being three and one-tenth days.

The usual signs of uremia were noted—anorexia, loss of weight, coma, convulsions and death.

In none of these animals was there a significant demonstrable rise in blood pressure (fig. 1).

Necropsy Observations.—Only in 2 animals were the changes remarkable. In one there was extensive lobular pneumonia, and in the other, a multiplicity of small hemorrhages throughout the gastrointestinal tract. Grossly as well as microscopically, the findings in the liver, heart and placenta were not remarkable, and the remaining organs were normal.

Nonpregnant Rabbits with Bilateral Nephrectomy.—The 4 nonpregnant females in which bilateral nephrectomy had been performed survived the operation on an average of four and a half days.

The signs and symptoms did not vary from those of the pregnant group in which nephrectomy had been performed.

No elevation of the systolic blood pressure was noted.

No necroses of the liver or heart were noted either grossly or microscopically.

COMMENT

Constriction of the renal arteries to a severe degree in rabbits, as in the other experimental animals, produces acute hypertension, albuminuria, hematuria, uremia, coma, convulsions and death. Constriction of the renal arteries to a minimal or a moderate degree, without notable renal lesions, produces hypertension, and the animals are clinically asymptomatic. Such variations are dependent on the degree of renal constriction. Pathologically, one finds multiple infarctions of the kidney and at times true cortical necrosis but rarely infarction of a whole kidney where a severe degree of constriction has been applied. Focal areas of injury in the myocardium, liver and intestines do occur.

The pregnant animal differs from the nonpregnant one in that coma, convulsions and death can be effected in it with less severe renal injury, and, significantly, more extensive, more frequent hepatic injury is demonstrated. When renal injury is minimal, the pregnant animal does not seem to differ from the nonpregnant animal, but there is a high percentage of premature offspring or of young stillborn at term (table, experiment 1 C). The factor of operative manipulation in such instances is difficult to evaluate, and as yet has not been adequately controlled.

It seems likely that the symptoms of these animals are referable to acute hypertension with terminal cardiac failure when renal constriction is of moderate degree and that when the renal damage is severe the symptoms are due in addition to renal insufficiency with terminal uremia.

The necrosis of the liver, heart and other organs may be explained on the basis of ischemia as a result of arteriolar narrowing. Goldblatt [5e] first demonstrated arteriolar lesions with focal necrosis in dogs with severe constriction of the renal arteries, and Wilson and Pickering [4] have recently described this occurrence in rabbits. Our observations in dogs substantiate those findings.[8]

The severity of the hypertension and of the focal tissue injury of other organs cannot be directly correlated with the amount of renal damage. The absence of significant lesions in control animals comparable with the pregnancy group tends to emphasize that the symptoms and pathologic changes are not associated with pregnancy or with renal insufficiency alone; they do suggest hypertension, renal injury and as yet unknown factors of pregnancy as necessary in their production.

Although our estimation of the constriction of the renal arteries is not quantitative, the failure to produce any significant variation of reaction in pseudopregnant, spayed and normal animals suggests that the corpus luteum is not the predisposing factor in the variation of response shown by pregnant animals.

It is of interest to compare the experimental observations when the dog is used as the test animal under like conditions of pregnancy and hypertension. Hepatic lesions have been demonstrated in the dog, as previously recorded; but such widespread hepatic lesions as occur in the rabbit are infrequently demonstrated in the dog. Differences in susceptibility to the experimental test of pregnancy plus hypertension may account for the variations in the demonstrated reaction in the dog and the rabbit. In the dog, as in the rabbit, long-standing or chronic hypertension may have little effect on the course of the subsequent pregnancy or of multiple pregnancies. Our observations when the dog was used as the test animal will be recorded in a subsequent communication.

Dieckmann and Brown [9] and others have classified the toxemia of pregnant patients clinically into pre-eclampsia, essential hypertension and cardiovascular renal disease. However, a study of the fatal cases of toxemia of pregnancy in Duke Hospital and in the department of pathology of Duke University is in agreement with similar reported pathologic studies in showing no comparable, well defined pathologic separation of the cases. As is well known, the degree of cardiorenovascular involvement and the presence or absence of the classic features of eclampsia are frequently at variance with the clinical picture. Thus, it has been suggested that eclampsia is a phase of arteriolonephrosclerosis and that the clinical syndromes represent variations of a single disease process.

8. Erickson, C. C., and Dill, L. V.: Am. J. Path. **15**:621, 1939. Dill and Erickson.[6]

9. Dieckmann, W. J., and Brown, I.: Am. J. Obst. & Gynec. **36**:798, 1938.

Many investigators have shown the close correlation between renal involvement and the toxemia of pregnancy. Stander [10] described a phase of the toxemic syndrome classified as the low reserve kidney. Zimmerman and Peters [11] wrote extensively of renal disease in cases of toxemia of pregnancy. Persistent hypertension, impairment of renal function and abnormal urinary findings have been noted in a significantly high percentage of cases of toxemia of pregnancy.[12] Renal changes have been demonstrated with frequency in patients dying in the acute stages of eclampsia.[13]

The many similarities between acute arteriolonephrosclerosis and at least some of the forms of toxemia of pregnancy are recognized, and this has suggested the possibility of a similar mechanism in the production of the hypertension. Goldblatt [5e] and others have expressed the belief that the "malignant phase of hypertension" produced in dogs by severe constriction of the renal arteries is analogous to the disease in man that has been classified as essential or malignant hypertension and acute arteriolonephrosclerosis. These authors and others [5] have shown that at least one mechanism for the production of hypertension is constriction of the renal arteries with the resultant renal ischemia. In our experiments on rabbits we have utilized a similar method of producing renal injury or ischemia to initiate the increase in blood pressure. The pertinent and significant added factor in our investigations is pregnancy. It is well to emphasize that pregnancy is the single indisputable common factor of eclampsia and the toxemias of like nature in man. In the experimental production of the malignant phase of hypertension it has been suggested that there is a "hypothetical effect substance" associated with, or producing by its action, the "mechanical factor" (elevation of blood pressure) and a "humoral factor" (renal insufficiency).[5e] It seems logical that similarly we may hypothesize a hypothetic "pregnancy factor" acting in association with, or enhancing the effect of, "the mechanical factor" and "the humoral factor" to produce the experimental lesions and response demonstrated. In like manner Page and Ogden,[14] in a consideration of hypertension in eclampsia, concluded "the vascular pathology of eclampsia requires the postulate of a circulating factor which behaves as a vascular toxin." Byrom [15] produced lesions of the kidney, liver and other organs in rats by the injection of pitressin and suggested that the lesions of eclampsia may be the result of vascular spasm

10. Stander, H. J.: Medicine **8**:1, 1929.
11. Zimmerman, H. M., and Peters, J. P.: J. Clin. Investigation **16**:397, 1937.
12. Peckham, C. H., and Stout, M. L.: Bull. Johns Hopkins Hosp. **49**:225, 1931. The Late Results of Eclampsia, editorial, J. A. M. A. **113**:941, 1939.
13. Bell, E. T.: Am. J. Path. **8**:1, 1932.
14. Page, E. W., and Ogden, E.: Am. J. Obst. & Gynec. **38**:230, 1939.
15. Byrom, F. B.: J. Path. & Bact **45**:1, 1937.

in a similar manner. Hofbauer [16] proposed that eclampsia be considered as due to an excessive secretion of the posterior lobe of the pituitary gland.

The experimental observations which we have recorded suggest that eclampsia may be a fulminating disease process produced by association of renal vascular disease and pregnancy, pregnancy acting as the additive incitant to produce the acute episode, toxemia of pregnancy. In summary we can state that hypertension and renal-vascular injury are realized in our experiments as in human toxemias and that the pathologic changes are comparable. That renal insufficiency is a major factor in the animal experiments is apparent. In human diseases this "humoral factor" may be brought about in another manner, as yet not elucidated. The albuminuria of the toxemias of human pregnancy may well be a reflection of this factor.

SUMMARY

Severe renal ischemia in the rabbit produces hypertension, albuminuria, uremia, convulsions and death. Focal necroses of the myocardium and necroses of the liver occur.

The pregnant rabbit differs from the nonpregnant one in showing increased susceptibility to the effects of renal ischemia or renal injury. The pregnant rabbit demonstates extensive focal necroses of the liver in a significantly high percentage of experiments.

Renal insufficiency alone or pregnancy alone does not account for the variation of response by the pregnant animal. Other endocrine states do not demonstrate the reaction of the pregnant animal. Hypertension, renal ischemia or renal injury in association with pregnancy seems to be the essential combination of factors.

These experimental observations support the thesis suggested in our report of preliminary observations that renal ischemia or renal injury in the pregnant animal produces a syndrome with involvement of physiologic factors similar to, and production of pathologic lesions analogous to, those in at least some of the human toxemias of pregnancy.

16. Hofbauer, J.: Am. J. Obst. & Gynec. **26**:311, 1933.

EXPERIMENTAL ATHEROSCLEROSIS

II. EFFECT OF THIAMINE HYDROCHLORIDE AND ASCORBIC ACID ON EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

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With few exceptions, the greater part of investigative work on the relation of the vitamins to vascular sclerosis has been on the production of arteriosclerosis in animals by excessive doses of irradiated ergosterol.[1] Menten and King [2] found that guinea pigs maintained on diets deficient in ascorbic acid and given injections of sublethal amounts of diphtheria toxin showed diffuse hyperplastic arteriosclerosis in the lungs, liver, spleen and kidneys. Westenbrink [3] found no difference in the cholesterol content of the brain or blood in normal and in polyneuritic animals; the blood cholesterol was normal in the incipient stages of thiamine deficiency, rose in the intermediate stage and returned to normal with the development of acute symptoms of polyneuritis. Sure, Kik and Church [4] reported no change in the blood cholesterol of rats with thiamine deficiency, and Takata [5] failed to notice any relationship between avitaminosis and the total cholesterol in pigeons. Recently, Member and Bruger [6] reported that thiamine hydrochloride given to rabbits with herpes simplex encephalitis did not influence the blood cholesterol.

In the present study, massive doses of thiamine hydrochloride and ascorbic acid, alone or in combination, were injected into rabbits being fed cholesterol. The effect of these vitamins on the cholesterol content of the blood and on the degree of cholesterol atherosclerosis in the aorta was determined.

From the Department of Medicine, New York Post-Graduate Medical School and Hospital, Columbia University.

1. Pfannenstiel, W.: Lancet **2**:845, 1928.
2. Menten, M. L., and King, C. G.: J. Nutrition **10**:141, 1935.
3. Westenbrink, H. G. K.: Arch. néerl. de physiol. **19**:122, 1934.
4. Sure, B.; Kik, M. C., and Church, A. E.: Proc. Soc. Exper. Biol. & Med. **29**:848, 1932.
5. Takata, Y.: J. Biochem. (Jap.) **24**:153, 1936.
6. Member, S., and Bruger, M.: J. Biol. Chem. **128**:lxix, 1939.

EXPERIMENTS

Two series of experiments were carried out. In the first of these, 25 rabbits varying in age from 3 to 5 months were used. Ralston-Purina chow[6a] was fed once daily, and each animal received 1 Gm. of cholesterol, mixed with the food, three times a week. On the days cholesterol was fed, the rabbits were given an intramuscular injection of physiologic solution of sodium chloride, thiamine hydrochloride or ascorbic acid in the doses indicated in the following list. Twenty of these rabbits survived the total experimental period of one hundred and one days and were divided into the following groups:

1. Four rabbits: 1 cc. of 0.9 per cent sodium chloride
2. Four rabbits: 5 mg. of thiamine hydrochloride (0.25 cc. of an aqueous solution)
3. Three rabbits: 50 mg. of thiamine hydrochloride (2.5 cc. of an aqueous solution)
4. Five rabbits: 5 mg. of ascorbic acid (0.25 cc. of an aqueous solution buffered with tenth-molar sodium phosphate)
5. Four rabbits: 50 mg. of ascorbic acid (2.5 cc. of an aqueous solution similarly buffered)

Twenty-four rabbits were obtained for the second series. The age of the animals, the basal diet and the cholesterol feedings were identical with those described for the first series. The injections were given three times a week on the day of the cholesterol feedings. Twenty of these survived the experimental period of sixty days and were divided into the following groups:

6. Three rabbits: No injections
7. Four rabbits: 1 cc. of 0.9 per cent sodium chloride
8. Six rabbits: 10 mg. of thiamine hydrochloride (1 cc. of an aqueous solution)
9. Three rabbits: 10 mg. of ascorbic acid (1 cc. of an aqueous solution buffered with tenth-molar sodium phosphate)
10. Four rabbits: 5 mg. of thiamine hydrochloride (0.5 cc. of an aqueous solution) and 5 mg. of ascorbic acid (0.5 cc. of an aqueous solution buffered as described)

The whole blood cholesterol of each rabbit was determined every fourteen to twenty-one days by a modification of the Bloor method.[7] The procedure used for killing the rabbits, the technic for removal of the aorta and the determination of its cholesterol content have been described elsewhere.[8]

RESULTS

In order to conserve space and because of lack of significant results, the detailed variations in the blood cholesterol in the two groups of experiments are not given (the average values at the termination of each

6a. This product is prepared by the Ralston Purina Company, St. Louis, and contains a mixture of grains and alfalfa hay, supplemented by vitamins and minerals.

7. Sackett, G. E.: J. Biol. Chem. **64**:203, 1925. Mirsky, I. A., and Bruger, M.: J. Lab. & Clin. Med. **18**:304, 1932.

8. Bruger, M., and Fitz, F.: Arch. Path. **25**:637, 1938.

series are noted in the table). After thirty-five to forty days had elapsed during the cholesterol feeding in the first series, it appeared that those rabbits receiving thiamine hydrochloride or ascorbic acid and cholesterol failed to show as high blood cholesterol as those receiving cholesterol alone. For this reason, the sixty day series was planned, but the subsequent study failed to confirm this impression. It may be safely stated that thiamine hydrochloride and ascorbic acid do not influence the blood cholesterol of rabbits fed cholesterol, at least in the doses used in this investigation.

The table gives the range and the average of the cholesterol contents of the aorta in the control and the experimental groups in both series of

Cholesterol Contents of the Aortas of Cholesterol-Fed Rabbits Receiving Injections of Sodium Chloride, Thiamine Hydrochloride or Ascorbic Acid

Group	Experiment	Rabbits	Blood Cholesterol, Mg. per 100 Cc.*	Cholesterol Content of Aorta, Gm. per 100 Gm. Dry Weight	
				Range	Average
	101 day experiment				
1	Sodium chloride (0.9%)	4	761	0.63-4.85	2.66
2	Thiamine hydrochloride (5 mg.)	4	687	0.92-2.90	1.80
3	Thiamine hydrochloride (50 mg.)	3	745	1.00-6.09	2.98
4	Ascorbic acid (5 mg.)	5	755	0.77-2.55	1.55
5	Ascorbic acid (50 mg.)	4	696	0.66-3.26	1.65
	60 day experiment				
6	No injections	3	670	0.54-0.68	0.59
7	Sodium chloride (0.9%)	4	640	0.50-1.32	0.88
8	Thiamine hydrochloride (10 mg.)	6	654	0.55-0.74	0.65
9	Ascorbic acid (10 mg.)	3	849	0.74-0.87	0.80
10	Thiamine hydrochloride (5 mg.) and ascorbic acid (5 mg.)	4	788	0.51-1.64	1.08

* Each value in this column represents the average blood cholesterol content for a group at the termination of the experiment.

experiments. It may be observed that thiamine hydrochloride and ascorbic acid do not exert any significant effect on the amount of cholesterol deposited in the aorta of the cholesterol-fed rabbit.

SUMMARY

Thiamine hydrochloride and ascorbic acid neither prevent nor accentuate the rise of the blood cholesterol in rabbits fed cholesterol. Furthermore, these vitamins neither inhibit nor enhance cholesterol atherosclerosis of the aorta.

EFFECT OF PROGESTERONE ON GROWING CARTILAGE AND BONE IN IMMATURE GUINEA PIGS

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In continuation of investigations of the effect of various hormones on the changes of cartilage and bone taking place during growth and aging, the present study is concerned with the response of the skeletal tissues of growing guinea pigs to progesterone.

MATERIAL AND METHODS

Twenty guinea pigs, 10 females and 10 males, weighing 150 to 170 Gm. at the beginning of the experiment, received subcutaneous injections of progesterone in the form of proluton (Schering) in sesame oil on six days of the week for periods of one, two, four, six and eight weeks. In one half of the animals the dose given each time was 1 rabbit unit, and in the other half, 0.65 rabbit unit.

Frontal sections were prepared from the upper third of one tibia, whereas the knee joint of the other side, which had been left intact at autopsy, was sectioned in a sagittal direction. Longitudinal sections were also made of the chondro-osseous junction of several ribs.

GENERAL OBSERVATIONS

Sixteen animals gained weight in a normal manner, the weights rising from 150 to 395 Gm. during a period of eight weeks, which is according to expectation. At autopsy the long bones, as well as the ribs, appeared thinner and more delicate than ordinarily. The length of the tibia, taken with the aid of a caliper, was 4.05 to 4.2 cm. after eight weeks of treatment, which is about normal.

Three guinea pigs of a group of 4 showed, after six weeks of injections, a sudden drop in weight from about 340 to 250 Gm., without apparent cause. These animals were therefore killed six and a half weeks after the beginning of the experiments. No specific lesions indicating intercurrent diseases were found at autopsy. A female belonging to another group likewise failed to gain weight during two weeks' observation.

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine.

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MICROSCOPIC OBSERVATIONS

After progesterone had been administered for one or two weeks the *zone of endochondral ossification* of the upper part of the tibia was somewhat enlarged. The chondromucoid ground substance was scarce, and its fibrils were delicate. The cartilage cell rows had a regular structure. A single row of these cells contained, as a rule, 12 columnar and 4 hypertrophic cartilage cells. The columnar cells were thus slightly increased over the normal number of 10, whereas the number of hypertrophic cells was unchanged. The nonoriented, so-called resting cartilage cells were enlarged and sent out numerous branching processes. The ordinarily flat columnar cartilage cells took on a polyhedric or ellipsoid shape. This was particularly noticeable in the proximal parts of the cartilage columns. Both the nonoriented and, in particular, the enlarged columnar cartilage cells proliferated actively and frequently by mitosis. The hypertrophic cartilage cells were large, and less calcium salts were deposited at their periphery than is normal. The capsules of the hypertrophic cartilage cells were readily opened by capillaries advancing from the bone marrow. The demarcation between the layer of hypertrophic cartilage cells as a whole and the bone marrow was sharp.

If the injections were continued for a period of one month, the amount of cartilaginous ground substance in the growth zone was still small, although the matrix was slightly swollen; normally at this age the cartilaginous ground substance has begun to increase in amount and becomes dense and sclerosed. The cellular structure of the epiphysial disk did not differ very much from that seen after one or two weeks of treatment with progesterone. Therefore in appearance the epiphysial disk of guinea pigs which had been treated with progesterone for one month and weighed 250 to 285 Gm. was comparable to that of untreated animals which were about one month younger and which had a weight of 150 to 180 Gm.

After administration of progesterone for a period of two months, the guinea pigs weighed between 365 and 396 Gm. The zone of endochondral ossification in these animals was still of good medium width, whereas normally guinea pigs of similar age and weight already show narrowing of the epiphysial disks. The amount of cartilaginous ground substance in the epiphysial growth zone was only slightly increased as compared with that at earlier stages and much smaller than normal. It also failed to show the increasing hyalinization and sclerosis usually seen at this age. The cell count in a single cartilage cell row was only a little higher than normal, namely, 3 to 4 hypertrophic and 9 to 10 columnar cartilage cells. But the cells were conspicuously larger, and more cell rows were present than in corresponding control animals (fig. 1 *A* and *B*). The nonoriented and the columnar cartilage cells were still observed to proliferate, and here and there they underwent mitotic division, a condition not found at this age under ordinary circumstances. The hypertrophic cartilage cells, although more calcified than at the earlier stages, were larger and showed somewhat less calcification than those in normal control guinea pigs, and bone formation was likewise decreased. Furthermore, while in normal guinea pigs of corresponding age some cartilage cells showed retrogressive changes and the onset of the closure of the growth zones was indicated by the formation of osseous plugs, in animals treated with progesterone no atrophy or degeneration of single cartilage cells or of whole cell rows and no formation of osseous plugs was observed. Thus, after two months of treatment the microscopic appearance of the epiphysial cartilage of animals weighing then about 400 Gm. was comparable to that of untreated guinea pigs four to six weeks younger and weighing about 250 Gm.

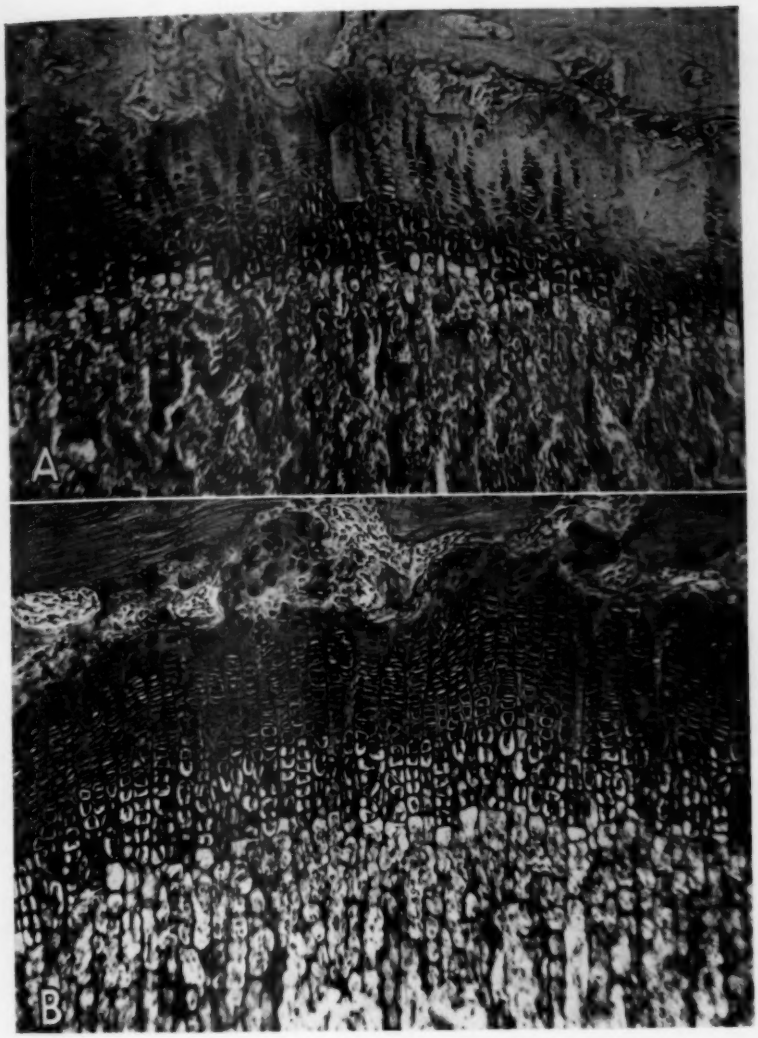

Fig. 1.—*A*, section through the epiphysial disk of the upper tibia of a normal female guinea pig whose weight during eight weeks of observation had risen from 150 to 405 Gm. Fairly large amounts of cartilaginous matrix are present; columnar cartilage cells are small and somewhat irregularly arranged. In the center of the disk an area of localized ossification is seen. Magnification, × 150.

B, section through the epiphysial disk of the upper tibia of a female guinea pig which had received 1 rabbit unit of progesterone six times weekly for a period of eight weeks. The weight of the animal had increased from 150 to 395 Gm. during this period. Little ground substance is present, and the cartilaginous disk is wider than in *A*. The columnar and hypertrophic cartilage cells are more numerous and larger than normally. The formation of subepiphysial trabeculae is lagging behind that seen in *A*. Magnification, same as in *A*.

The subepiphysial zone was wide and well vascularized. In the early stages relatively few osteoblastic cells were present, and their conversion into osteocytes took place at a slow rate. After one month of injections of progesterone, large osteoblastic epithelioid cells were more numerous. These proliferated mitotically and were lying close together in and around corroded cartilage cell capsules. Farther distally they were situated along the longitudinal processes, which were remnants of the calcified walls of corroded cartilage cell capsules. Thus trabeculae were formed. But, because little osseous ground substance was laid down, the conversion of the osteoblasts into dense and mature small osteocytes was delayed. A soft fibrocytic connective tissue interspersed with some bone marrow cells separated these thin and rather immature subchondral spicules from each other. Few osteoclastic giant cells were seen. Farther distally in the marrow cavity the bone marrow was cellular and contained fairly numerous but thin bony trabeculae, at the surface of which osteoblasts, as well as some osteoclastic giant cells, were present.

The findings described so far were observed in 16 of 20 guinea pigs. In 4 animals, which had lost or failed to gain weight, a reaction different from that seen in the majority of the animals was noted microscopically. These 4 had certain histologic features in common. The growth zones were narrowed. The cartilage cells were decreased in number and size. The cell count in a single cartilage cell row had decreased to 1 to 2 hypertrophic and 6 columnar cells. The cartilaginous matrix was increased in amount and dense, and a collagenous and preosseous layer of ground substance traversed the narrowed zone of the hypertrophic cartilage. The proliferation of the cartilage cells continued, and here and there mitotic division of columnar cartilage cells was seen. The subepiphysial layer was poorly vascularized and narrowed, and bone formation took place close to the zone of hypertrophic cartilage and was intensified as compared with conditions in those guinea pigs which, under the influence of progesterone, had not shown a loss in weight. The histologic appearance of the epiphysial zones of those guinea pigs which had lost weight resembled, therefore, that seen in underfed guinea pigs.[1] Yet the influence of the progesterone was recognizable to a certain extent, as the epiphysial cartilage cells were larger and more numerous and less bone was present than in underfed guinea pigs which had not been treated with progesterone. Atrophy and solution of the cells of the bone marrow also indicated that in these animals there might have been certain nutritional disturbances.

Joints.—After one or two weeks of injections of progesterone the ordinarily flat cells of the upper layer, composing the sliding zone, enlarged, rounded off and became more numerous. The cells of the transitional and pressure zones also increased in size and not infrequently showed mitotic proliferation. The cartilaginous ground substance was diminished in amount. The hypertrophic cartilage cells were less calcified than ordinarily; they readily broke down under the influence of elements of the bone marrow. The subchondral tissue contained large osteoblasts, the conversion of which into osteocytes was delayed. These changes became more accentuated four, and particularly eight, weeks subsequent to the beginning of the administration of progesterone. In the sliding and transitional zones numerous cells were present, which still proliferated here and there by way of mitosis. These cells were larger and the formation of cartilage capsules was much less distinct than usual (fig. 2*A* and *B*). Whereas in normal guinea pigs of corresponding age and weight the trabeculae consisted of dense and lamellated

1. Silberberg, M., and Silberberg, R.: Arch. Path. **30**:675, 1940.

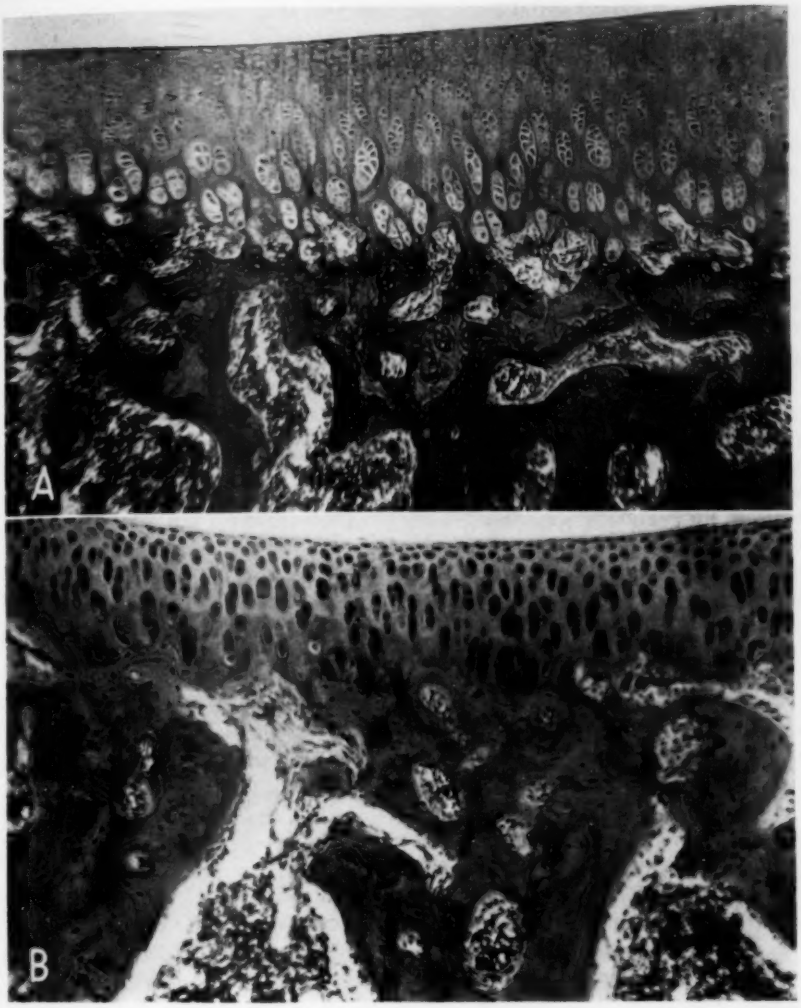

Fig. 2.—*A*, section through the upper articular surface of the tibia of a normal female guinea pig whose weight had risen from 150 to 380 Gm. during eight weeks of observation. The various cartilage cell layers can be readily distinguished. The bony trabeculae consist of dense ground substance and small osteocytes. Magnification, × 220.

B, section through the upper articular surface of the tibia of a female guinea pig which had received 0.65 rabbit unit of progesterone six times weekly for a period of eight weeks. The weight of the animal had increased from 160 to 385 Gm. during this period. Cells of the various layers are somewhat hyperplastic and hypertrophic. Cartilage capsules are less distinct than in *A* and there is less ground substance present. The bony trabeculae contain large osteocytes and soft ground substance. Magnification, same as in *A*.

bony substance in which small osteocytes were situated, under the influence of progesterone the subchondral trabeculae contained large osteocytes separated from each other by a good deal of soft osseous substance. Only small amounts of calcium had been laid down in the bony substance, and the lamellation of the latter was less sharp. The bony tissue therefore appeared rather immature.

In the 4 animals which had lost or failed to gain weight, the matrix of the articular cartilage was increased in quantity and sclerosed. The cartilage cells were smaller and less numerous and more bone had been produced in the subchondral layer than in those guinea pigs which had shown a steady gain in weight. Thus the conditions were comparable to those in the epiphysial zones of these particular guinea pigs, and the influence of the nutritional disturbance manifested itself also in the articular cartilage. But, as was the case in the growth zones, a certain effect of the progesterone was noticeable even here, as the number and size of cells were larger than in underfed animals which had not been treated with progesterone.

Bony Shaft.—The changes which took place in the compact bone of the shaft under the influence of progesterone were similar to those seen in the osseous trabeculae. Increasingly during the first four weeks, and still more so after two months of treatment, the compacta became less firm than ordinarily and showed wide haversian canals. The osteocytes and their nuclei were large, and between the bone cells were found fairly large amounts of a soft and only slightly calcified ground substance. The periosteal osteoblastic epithelioid cells which were in contact with the outer surface of the shaft were less numerous than normally; osteoclastic giant cells were even scarcer. The periosteal connective tissue was soft and cellular. No changes were noted in the cells lining the inner surface of the compacta.

In the guinea pigs which showed a loss or lack of gain in weight, the compact bone was denser and more intensely calcified. Likewise the periosteal connective tissue was dense and fibrillar, and the osteoblastic epithelioid cells were more numerous but smaller than those in the animals which had gained in weight.

Ribs.—After administration of progesterone the growth zone at the chondro-osseous junction of the ribs was enlarged. The columnar cartilage cells were more numerous and larger than ordinarily. The hypertrophic cartilage cells contained less calcium than normally. As was the case in the long bones, the osseous structures were less mature than those in control animals. Even as late as eight weeks after the beginning of the injections the cartilaginous ground substance was scarce and showed little sclerosis and hyalinization. The cartilage cells were better preserved than under normal conditions and manifested fewer retrogressive changes than is usual for this age.

In those guinea pigs which had not gained or which had even lost weight, the growth zone was rather narrow. The processes of calcification, hyalinization and sclerosis of the cartilage were increased, and more bone had been produced than in the animals which had gained weight steadily.

COMMENT

In growing guinea pigs, progesterone administered for periods up to two months exerts the following effects on cartilage and bone: (1) It slightly promotes the proliferation and increases the hypertrophy of the epiphysial and articular cartilage cells; (2) it inhibits retrogressive changes, calcification and ossification of the epiphysial cartilage, which

would normally occur during the period of observation, and it thus maintains the juvenile character of this tissue for an abnormally long period; (3) it inhibits to some extent the deposition of dense osseous substance and the conversion of immature osseous structures into mature bone; (4) in the ribs it inhibits atrophy, degeneration and calcification and thus counteracts the age changes of the costal cartilage.

These effects bear a certain similarity to those taking place in cartilage and bone subsequent to ovariectomy.[2] They differ from the latter particularly in that the active proliferation of the cartilage and the resorptive processes involving the bony structures are more pronounced in ovariectomized guinea pigs than in animals treated with progesterone.

As to the effect of progesterone on the cartilage, the slight hyperplasia and the increase in hypertrophy of the cartilage cells may be interpreted as actual stimulation of these cells by the administered substance. Besides this, however, there seems to be another and more pronounced effect, which consists in an inhibition of the normal processes of ossification and of aging.

While the stimulation of growth may be directly due to progesterone, there is also the possibility that this substance may act on the skeletal tissues through the intermediation of other endocrine glands. With reference to the inhibition of the age changes, it is conceivable that the antagonism which Loeb,[3] Courrier,[4] Allen and Meyer [5] and others have shown to exist between estrogen and progesterone in the uterus and vagina may in some way be involved in the action of progesterone on cartilage and bone. The changes in the skeleton observed subsequent to the administration of progesterone represent almost the opposite of the acceleration of the age changes which we found in the skeletal tissues following injections of estrogen.[6] An inhibition of the effect of estrogen, produced in the animals treated with progesterone, may partly account for the similarity of the findings subsequent to ovariectomy and to administration of progesterone. But while progesterone inhibits the effects of estrogen, ovariectomy eliminates them, provided other organs do not take over the production of the latter substance.

The duration of our experiments was comparatively short. We cannot, therefore, decide at present whether the effects exerted by progesterone on the skeleton are permanent or transitory or whether they

2. Silberberg, M., and Silberberg, R.: Am. J. Path. **16**:491, 1940.

3. Loeb, L.: J. Morphol. **22**:37, 1911; Am. J. Anat. **32**:305, 1923. Loeb, L., and Kountz, W. B.: Proc. Soc. Exper. Biol. & Med. **24**:728, 1927; Am. J. Physiol. **84**:283, 1928. Moore, E.; Smadel, J. E.; Koenig, K. F., and Loeb, L.: Growth **1**:119, 1937.

4. Courrier, R.: Compt. rend. Soc. de biol. **104**:280 and 1178, 1930.

5. Allen, W. M., and Meyer, R. K.: Anat. Rec. **61**:427, 1935.

6. Silberberg, M., and Silberberg, R.: Arch. Path. **28**:340, 1939.

may even be reversed at later stages, a condition which has been observed to occur subsequent to administration of bovine hypophysial extract and of thyroxin.[7]

SUMMARY

In growing guinea pigs, progesterone administered subcutaneously over periods of up to two months (1) increases slightly the proliferation and more markedly the hypertrophy of the euhyaline cartilage of the epiphysial zones, articular surfaces and ribs; (2) inhibits the retrogressive changes, calcification and sclerosis of the cartilage which normally occur with advancing age, and (3) inhibits the conversion of immature osseous substance into mature bone, thus counteracting the aging of bone.

No definite conclusions can as yet be drawn as to the mechanism underlying these changes—in particular, as to whether all or some of these changes are due to a direct effect of progesterone on the skeletal tissues or whether an intermediary action of other endocrine glands or hormones is involved.

7. Silberberg, M., and Silberberg, R.: Am. J. Path., to be published; Growth, to be published.

PULMONARY EMBOLISM AND INFARCTION

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DECATUR, ILL.

The encouraging success of thoracic surgery for pulmonary tumors and the improvement in the surgical treatment of tuberculosis of the lungs are important grounds for the newly awakened interest in pulmonary circulation. The experiments on pulmonary infarction which I shall describe will contribute, it is hoped, to further clarification of the purely anatomic problems connected with this subject. There is still some uncertainty as to the conditions leading to infarction in some cases and to its absence in other, though similar, cases.

EXPERIMENTS

Series 1.—Guinea pigs were anesthetized with ether, and then maintained by means of a McCurdy model safety gas-oxygen machine and an ordinary small nasal mask. A flow of approximately 88 per cent ethylene and 12 per cent oxygen was used, with the exhaling valve closed. By the time the incision was made and the lung exposed, the gas bag was well filled. By pressure on the bag, the lung was expanded so that the lobe slipped out of the thorax. After the operation the pressure was released, the flow of gas closed off and anesthesia maintained with the remaining mixture in the bag until the operation was completed. The time consumed was about seven minutes. The guinea pigs responded almost immediately; all of them survived the operation and were in good condition until killed.

In 5 guinea pigs the skin was removed from the thorax, and a hole about 1 cm. long was made with a blunt forceps in an intercostal space at the level of the heart. The lobe of the lung was forced out of the chest by the method described. A string was firmly tied around the lobe near the hilus and the wound closed by sutures.

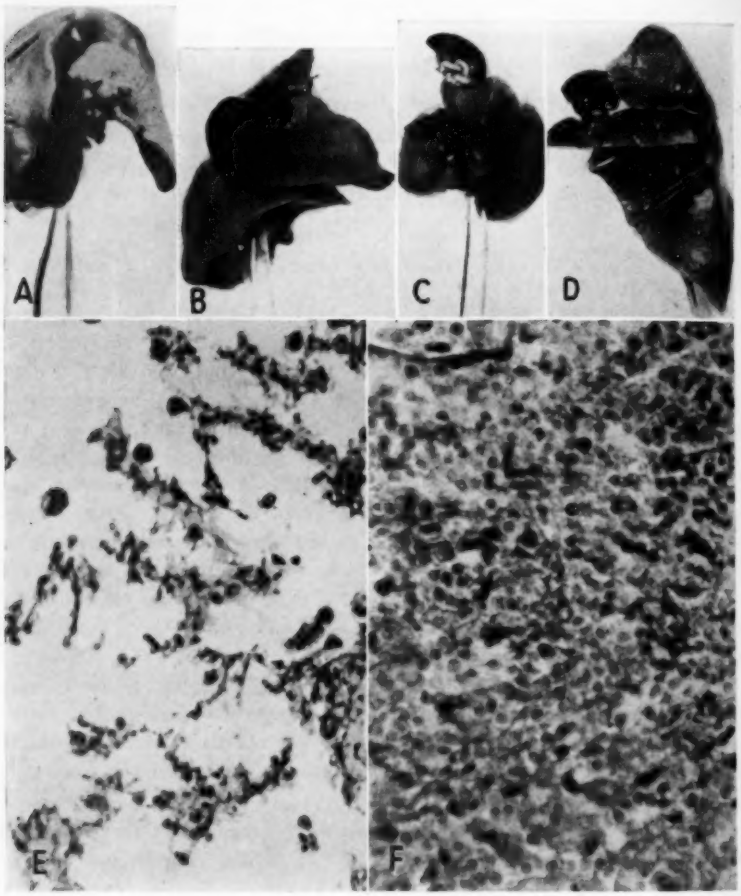
After twenty-four hours the ligated part of the lung was found to be anemic in all cases. It was pale, whitish, firm and had shrunk to about one third of its original size (*A* in figure). As compared with the next group, there was much fibrin around the necrosed portion. Microscopically, the shrunken part showed small, collapsed air spaces limited by disintegrating walls, the nuclei of which did not stain. With the hematoxylin-eosin stain, the field was uniformly pinkish except for a few white cells in the walls and within the air spaces (*E* in figure).

In 10 guinea pigs under the same conditions a string was loosely tied around a portion of a lobe, constricting the diameter to about one third of its original length. The wound was closed by sutures. All animals were killed while in good condition, twenty-four hours after the operation.

From the Pathological Laboratory of the Decatur and Macon County Hospital.

Seven of the animals showed a typical hemorrhagic infarction of the constricted area (*B*, *C* and *D* in figure). The infarcted portion was enlarged, hard, dry, black-red. There was a thin layer of fibrin on the pleura and but little fibrin around that area. The adjacent lung tissue on the opposite side of the string, toward the hilus, was atelectatic and hyperemic.

Microscopically, the infarcted area showed the air spaces filled with red blood cells, most of which were clumped. The walls of the air spaces were flooded



A, anemic necrosis in the lung of a guinea pig twenty-four hours after ligature; actual size.

B, *C* and *D*, hemorrhagic infarcts in lungs of guinea pigs twenty-four hours after a string was tied loosely around the lobe; actual size. The string is seen in *C*; in *D* the string has been removed to show the narrowing.

E, anemic necrosis in the lung of a guinea pig twenty-four hours after ligature, showing empty air spaces with disintegrated walls; × 400.

F, hemorrhagic infarction in the lung of a guinea pig twenty-four hours after the lobe had been tied loosely. The air spaces are filled with erythrocytes, obscuring the lung structure.

with red cells, obstructing the view of the walls. There was a small amount of serum in some air spaces. Veins and arteries alike were dilated, filled with and surrounded by clumped red cells (fig., *F*). The bronchi were narrowed with intact epithelium. Beneath the pleura, about one or two rows of air spaces showed less dense hemorrhage; the blood cells were isolated from one another. The region where the string was placed showed extreme atelectasis; just below the string was a narrow zone of necrosis.

In the 3 remaining animals, only atelectasis and hyperemia were found, and the string could be easily stripped from its location.

Series 2.—In this series rats were used and the infarcts were produced without opening the chest.

After the removal of the skin from the chest, the rat being under ether anesthesia, a curved needle, with suture attached, was introduced into the thorax nearly parallel to the sternum. A part of the lung, caught in this way, was ligated to the chest wall. In 15 of the 25 rats a hemorrhagic infarction developed, similar in appearance to that in the guinea pig. However, anemic necrosis could not be produced by this method.

Next an attempt was made to produce anemic, as well as hemorrhagic, infarcts in the kidneys by tying strings tightly or loosely around the hilar vessels or around the renal substance. While it was easy to produce anemic necrosis of the kidney by tying the artery or the hilar vessels, hemorrhagic infarction of the kidney could not be produced. Severe hyperemia, grossly resembling an infarction, with occasional petechiae, was seen, but the microscopic picture of an infarct with the structure obliterated by red cells was not found.

COMMENT

In order to understand the genesis of pulmonary infarcts, a short consideration of pulmonary embolism as far as changes in the lung tissue are concerned seems advisable.

In man, occlusion of the *stem* of the pulmonary artery occurs usually as a result of the lodgment of an embolus from the large vein of a leg. Death ensues as a result of a rapid fall in arterial pressure and an equal rise in venous pressure if this change, according to Hall and Ettinger,[1] lasts longer than ninety-three seconds. Because of lack of time, no severe tissue changes in the involved lung can be noted.

There are, however, cases, "not infrequently" met with (Gibbon, Hopkinson and Churchill,[2] also de Takáts and Jesser [3]), in which patients with pulmonary embolism live apparently long enough, up to twenty-four hours and longer, for some changes to be produced. Lungs from patients with congenital pulmonary stenosis or occlusion are normal (Christeller [4]).

1. Hall, G. E., and Ettinger, G. H.: Canad. M. A. J. **28**:357, 1933.

2. Gibbon, J. H., Jr.; Hopkinson, M., and Churchill, E. D.: J. Clin. Investigation **11**:544, 1932.

3. de Takáts, G., and Jesser, J. H.: J. A. M. A. **114**:1415, 1940.

4. Christeller, E.: Virchows Arch. f. path. Anat. **223**:40, 1916; cited by Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, W. de Gruyter & Co., Berlin, 1928, vol. 2, p. 291.

In dogs living up to three hours after embolization of the main pulmonary artery, Mendlowitz [5] found only congestion.

Ligature of the *artery to one lung only* produces at first mild stasis and hyperemia; then, in months or years, shrinkage of the lung with mild fibrosis. Schlaepfer [6] found no necrosis or infarction.

Occlusion of a *medium-sized* branch (second or third order) of the pulmonary artery represents one of the oldest of pathologic experiments.[7] While most of those who have reported it succeeded in producing an infarct, all have agreed that it is not easy to do this and that only in a minority of cases does simple occlusion of a branch of the pulmonary artery produce an infarct.

In résumé, the occlusion of the pulmonary artery at any point of its course leaves the lungs intact as far as severe changes of tissue are concerned.

There are exceptions to the rule. Schlaepfer [6] reported an instance in which the pleural cavity was filled with a caseous mass and no trace of lung tissue was left. Mathes, Holman and Reichert [8] reported necrosis in 4 of about 20 dogs with ligature of the pulmonary artery to one lobe of the lung and attributed the necrosis to infection. It is well, I believe, to assume that such a lesion represents genuine primary necrosis after ligature, with or without infection.

There are records in human pathology (Schmidt [9]) of anemic infarcts, small whitish wedge-shaped areas the size of an ordinary infarct, in noncongested lungs, representing anemic necrosis. This is obviously analogous to what may sometimes happen to a whole lobe.

There is no explanation available why in exceptional cases anemic necrosis follows occlusion of the pulmonary artery—in other words, why no collateral circulation takes place in these cases.

It is generally accepted that most hemorrhagic infarcts in human lungs are encountered when and where there is increased venous pressure.

Although it is possible to produce experimental hemorrhagic infarcts in normal lungs by simple embolization of branches of the pulmonary

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5. Mendlowitz, M.: J. Thoracic Surg. **8**:204, 1938.

6. Schlaepfer, K.: Arch. Surg. **9**:25, 1924; **13**:623, 1926.

7. (*a*) Cohnheim, J., and Litten, M.: Virchows Arch. f. path. Anat. **65**:99, 1875. (*b*) Litten, M.: Ztschr. f. klin. Med. **1**:131, 1880. (*c*) Orth, J.: Centralbl. f. allg. Path. u. path. Anat. **8**:859, 1897. (*d*) Fujinami, A.: Virchows Arch. f. path. Anat. **152**:61, 1898. (*e*) Zahn, X.: Centralbl. f. allg. Path. u. path. Anat. **8**:860, 1897. (f) Karsner, H. T., and Ash, J. E.: J. M. Research **27**:205, 1912-1913. (*g*) Steinberg, B., and Mundy, C. S.: Arch. Path. **22**:529, 1936.

8. Mathes, M. E.; Holman, E., and Reichert, F. L.: J. Thoracic Surg. **1**:339, 1932.

9. Schmidt, B.: Beitr. z. path. Anat. u. z. allg. Path. **99**:131, 1937.

artery, Orth [7e] found it easier if he compressed the lungs two days after introduction of mercury into the jugular veins. Steinberg and Mundy [7g] obtained infarcts with single lead shots, but their results were more regular if they introduced hundreds of lead shots, instead of one. Occlusion of numerous small branches in all probability produces acute stoppage of the outflow of blood from the heart, with a rise of venous pressure.

In the experiments described in this paper, increased venous pressure was obtained by putting a string around a lobe and tying it not too tightly, for fear anemic necrosis might ensue. Infarcts were obtained in 22 of 35 animals. This seems a fair percentage (63 per cent).

The experiments indicate that anemic infarcts do not develop from the hemorrhagic. In twenty-four hours an area entirely deprived of its blood supply is pale and white, or just slightly pinkish. A few animals were observed for four days after the production of hemorrhagic infarcts and, if anything, the involved areas were darker red-black than on the first day. Karsner and Ash [7f] noted beginning discoloration after a week or so.

The lungs have a double blood supply, through the pulmonary artery and through the bronchial arteries. According to Miller,[10] in his extensive studies the lung parenchyma, air spaces and finer alveolar ducts were found supplied by the pulmonary artery and the bronchi by the bronchial arteries. Bronchi and lung tissue proper have separate blood supplies, but there are capillary (and only capillary) anastomoses between these two territories in normal lungs.

If the pulmonary artery is occluded at any point, the bronchial arteries as a rule act as collaterals. If the occlusion has been of long enough duration, a restoration of the circulation of the pulmonary artery is accomplished by not only capillary but larger anastomoses between bronchial and pulmonary branches. Vascular anastomoses have been demonstrated by Woods,[11] Miller,[10] Mathes, Holman and Reichert [8] and Steinberg and Mundy.[7g]

The experiments on kidneys were undertaken to produce at will anemic or hemorrhagic infarcts in one more organ as had been done in the intestine [12] and lungs. Unfortunately, the experiments on the kidneys failed. Anemic necrosis has been produced numerous times before and was easily reproduced by ligature of the renal artery. Hemorrhagic infarcts are practically unknown in any kidneys except those of desiccated young infants, in which bilateral thrombosis of the renal veins

10. Miller, W. S.: The Lung, Springfield, Ill., Charles C. Thomas, Publisher, 1937.

11. Wood, H. C.: J. Exper. Med. **14**:326, 1911.

12. Loeffler, L.: Arch. Path. **28**:323, 1939.

may give a picture similar to that of a hemorrhagic infarct. A string was put not only around the vessels, in different modifications, but also around the substance of the kidney. It was apparently impossible to tie the string loose or tight enough.

If blood stops entering an area, anemic necrosis follows. If blood continues to enter and comes to stay under high pressure, hemorrhage follows. The occlusion of a vein in the absence of sufficient collaterals produced a picture more like an infarct than the occlusion of an artery. A hemorrhagic infarct therefore can be said to represent the highest degree of passive congestion.

High pressure is not the only reason for hemorrhage; any toxic drug can cause this. But under the conditions in question increased venous pressure seems to be the only reason that a hemorrhagic instead of an anemic infarct occurs commonly in the lungs. However, both the anemic and the hemorrhagic infarcts are exceptional, as must be concluded from experiments and the literature. As a rule, changes do not occur, because of the collateral circulation through bronchial arteries.

SUMMARY

In guinea pigs and rats, putting a string around a lobe of the lung moderately tightly produced hemorrhagic infarction. Tying a string suddenly and tightly around a lobe produced anemic necrosis.

Stoppage of the blood supply to the kidneys, liver, lungs and intestines, to summarize the present and previous experiments, produced anemic necrosis, an observation that might be applied to any organ.

A hemorrhagic infarct, wherever it occurs, requires a continuous blood current and increased venous pressure. The continuous blood flow is maintained either by collaterals, as in the lungs, or by incomplete occlusion of the arteries, as in the intestines.

Occlusion of the pulmonary artery at any point of its course does not, as a rule, cause serious disturbance of the lung tissue, because there is sufficient collateral circulation through the bronchial arteries. The anemic and the hemorrhagic infarct in lungs represent exceptions to this rule, due to special conditions.

Case Reports

CONGENITAL HÜRTHLE CELL TUMOR

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General Director of Laboratories, Department of Hospitals, City of New York

The derivation of the so-called Hürthle cell tumor is obscure, and for present purposes perhaps the less said of it the better. In certain quarters the cell of origin is regarded as a thyroid unit; in others, as a parathyroid derivative. By some it is looked on as a phase in the secretory activity of thyroid epithelium; by others, as a product of degeneration. Whatever the truth may be, the tumor arising from this cell is divisible into two varieties—one, an adenoma showing no malignant qualities other than those traceable to the effects of pressure; the other, a tumor displaying the ability to metastasize, thus falling into the category of a malignant neoplasm.

I have had occasion to observe a Hürthle cell tumor removed at operation from an infant 6 weeks of age that had been present at the time of birth. The occurrence of this form of growth at this period of life is believed not previously to have been recorded.

REPORT OF A CASE

The patient was a girl 6 weeks of age, who was admitted to Bellevue Hospital, April 19, 1940. The family history was irrelevant.

The patient presented a large swelling in the region of the thyroid gland. It had been present since birth, and had been growing progressively larger. The growth consisted of three nodular masses—one in the midline, corresponding to the position of the isthmus of the thyroid gland, and one on either side, representing, apparently, involvement of the lateral lobes. The patient was in obvious respiratory distress, and this became so alarming that an emergency operation was performed by Dr. Fenwick Beekman four days after the patient's admission to the hospital. The growth was removed in two sections—one, rounded and irregularly lobulated, measuring 6 by 3 by 1.5 cm.; the other, likewise lobulated, measuring 5 by 3.5 by 2 cm. Both masses were grayish red and firm in consistency, and were partially encapsulated by connective tissue. The cut surface was smooth and glistening and similarly colored (fig. 1). That portion of the growth which lay in the midline extended through the upper aperture of the thorax and, for the greater part, was not surgically accessible. At the time of operation it was noted that the trachea was displaced in a backward direction. It was estimated that about four fifths of the growth had been eradicated. The total weight of tissue removed was 47 Gm.

After operation the patient continued to show signs of respiratory embarrassment, especially during inspiration, and was occasionally cyanotic. She was discharged from the hospital June 19, improved.

From the Laboratories of Pathology, Bellevue Hospital.

Microscopic Examination.—Numbers of sections were prepared from different parts of both masses. No tissue such as is customarily observed in the thyroid gland was identifiable.

The growth consisted of a connective tissue capsule from which vascularized trabeculae penetrated in various directions, dividing the tumor into lobules of different shapes and sizes made up of alveolar formations. The better formed alveoli were lined by a single layer of large pale cells with relatively small, rather

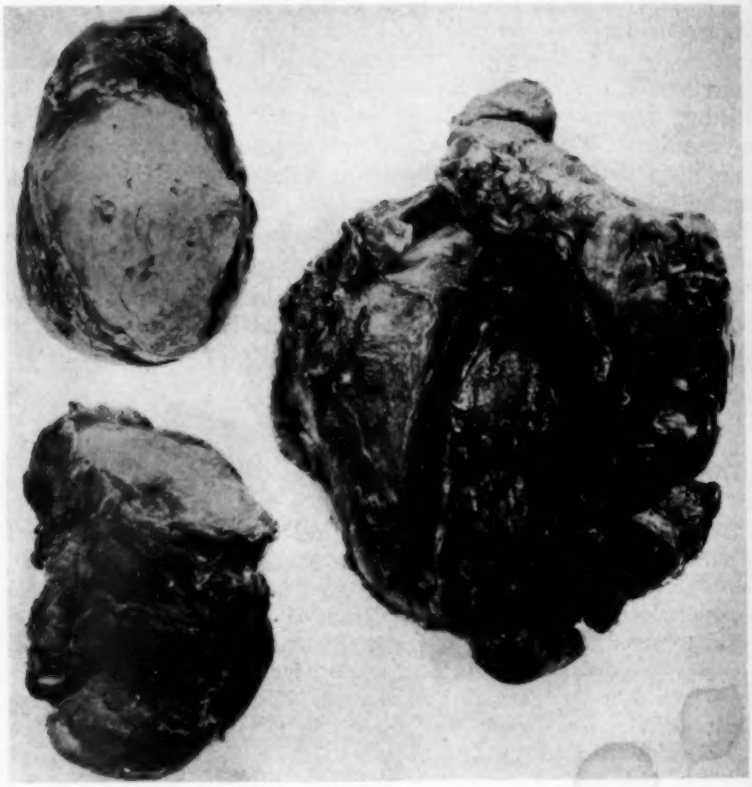

Fig. 1.—Photograph of the two masses removed at operation. The mass at the left has been divided to show the appearance of the cut surface.

dense, sometimes finely stippled, rounded nuclei variously placed in the body of the cell, often eccentrically, and with hyaline or finely granular, slightly acidophilic cytoplasm, the whole resting on a thin membrane provided with elongated nuclei, the membrane serving as a dividing septum between adjacent alveoli (fig. 2*A*). Intracellular fat-free vacuoles were numerous (fig. 2*B*). In many instances the alveoli were partly or completely filled with desquamated cells of the same type as those described.

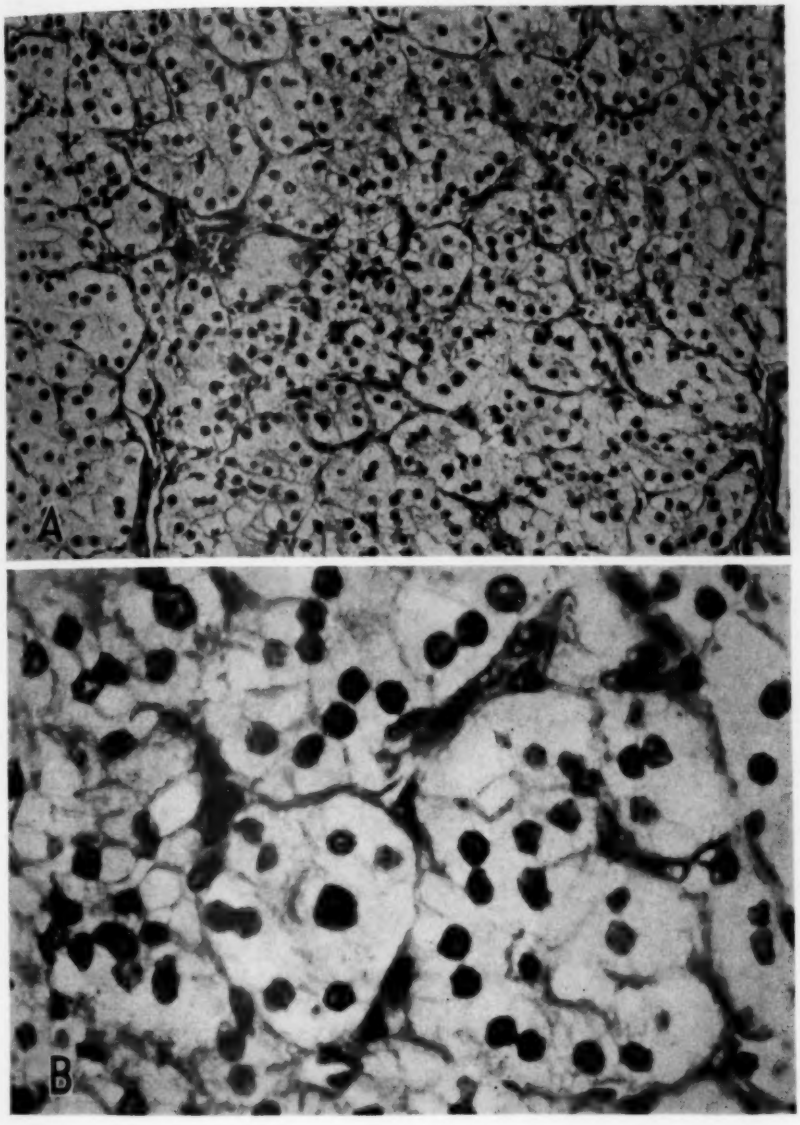

Fig. 2.—*A*, low power photomicrograph of the congenital Hürthle cell tumor described in the text, showing vascularized fibrous trabeculae and large, pale, vacuolated (fat-free) cells arranged in alveoli. *B*, high power photomicrograph of the same tumor shown in *A*, emphasizing the vacuolated (fat-free) cells.

COMMENT AND SUMMARY

There is a Hürthle cell tumor which is of congenital origin. Its ultimate cell derivation, like that of the same sort of growth occurring in later life, is not apparent—whether thyroid, parathyroid or otherwise.

In my experience intracellular fat-free vacuoles such as were found in the congenital Hürthle cell tumor here described are not encountered either in the normal thyroid or in its neoplastic descendants. Whether these cells bear any relation to the fat-free vacuolated cells met with in the parathyroid is not clear.

The congenital Hürthle cell tumor now recorded appears histologically to be an adenoma, but in view of the fact that its description is based on tissue removed surgically one cannot deny the possibility of malignancy and of the presence or potential occurrence of secondary growths in regional or remote areas.

The congenital Hürthle cell tumor here described seems to be unique inasmuch as no account of a comparable growth could be found in the literature on medicine.

STAPHYLOCOCCIC ACTINOPHYTOTIC (BOTRYOMYCOTIC) ABSCESS OF THE LIVER WITH PULMONARY INVOLVEMENT

A. A. Fink, M.D., Cincinnati

The term "staphylococcic actinophytosis" refers to a granulomatous lesion resulting from Staphylococcus aureus infection. The lesion is a slowly progressive granulomatous abscess varying considerably in its morphologic aspects from the lesion ordinarily produced by Staph. aureus. This variation is apparently due to a peculiar state of balance between the dose and virulence of the bacteria, on one hand, and the local and general resistance of the host, on the other. The process has more commonly been spoken of as botryomycosis, but because of the use of this term by Karsner [1] and certain dermatologists to refer to a telangiectiform infected lesion of the skin, "staphylococcic actinophytosis," as suggested by Berger [2] and others, is preferable. Less commonly, the descriptive term "granuloma pyogenicum" has been used to refer to this lesion. The term "actinophytosis," referring to the peculiar state of balance between the host and the micro-organism, may also be applied to lesions initiated by bacteria other than Staph. aureus which show the same ability to attain such a state.

REPORT OF CASE

A white boy 16 years old was admitted to the Cincinnati General Hospital, Dec. 16, 1938, with a history of chilliness and weakness for two weeks, nonproductive cough for one week and nausea and vomiting for three days. Examination revealed bilateral pleural effusion, general enlargement of lymph nodes, mild disseminated furunculosis of the skin and secondary anemia. There continued to be afternoon fever. December 18, a deep subcutaneous abscess of the right buttock was recognized, and from this lesion an organism was cultured which was first reported to be Staphylococcus albus; later the infectious agent was reported to be Staph. aureus, although a poor color producer. The boy continued to have fever ranging between 100 and 103 F., and Jan. 5, 1939 he was first given staphylococcus toxoid. This treatment was without evident effect. January 12, he developed a paronychia of the right thumb. March 5, still febrile, he was released from the hospital to be followed in the outpatient dispensary. May 2, it was recorded that he had had four furuncles on the buttocks during the past month.

May 19, the patient was readmitted with an account of severe cough and sore throat for the past eleven days, drenching sweats, and pain in the right side of the chest for the past five days. Physical examination and roentgen studies revealed

From the Department of Pathology, University of Cincinnati, and the Cincinnati General Hospital.

1. Karsner, H. T.: Human Pathology, Philadelphia, J. B. Lippincott Company, 1926, p. 286.

2. Berger, L.; Vallée, A., and Vézina, C.: Arch. Path. **21**:273, 1936.

pleural thickening in the right side of the chest and a confluent infiltrate in the lower lobe of the right lung. A diagnosis of pneumonia of the lower lobe of the right lung was made, with some uncertainty; no pneumococci were obtained from the sputum or from the blood culture. July 10, iodized poppyseed oil was instilled into the bronchi, and a diagnosis was made of bronchiectasis of the lower lobe and incomplete expansion of the middle lobe of the right lung. The patient began to show purpuric lesions on the legs, and blood was noted in the urine and feces August 9. September 14, following a series of blood transfusions, it was thought that there was improvement, although hematuria persisted. The boy was again released, September 24, although still febrile and with his physical condition unchanged. He was followed in the outpatient dispensary, where, October 20, he presented himself with numerous furuncles over his face, signs of active nephritis and severe anemia (red blood cell count 1,740,000 and hemoglobin 7.5 Gm.). He refused to reenter the hospital.

On his final admission, November 7, he gave an account of chills, fever, weakness and vomiting for the past two days. The urine had shown a dark color for two weeks, and there had been nocturia. He had noted also the reappearance of red spots on the feet in addition to recurrent swelling of the face, abdomen and ankles. Examination showed generalized icterus, petechiae and purpura of both legs and pustules and acne of the face and shoulders. Breath sounds were absent over the lower two thirds of the right side of the chest. The icteric index was 35. Fluoroscopic study, November 11, showed an enormous liver, a large spleen, ascites and pleural thickening over the base of the right lung. Aspiration of the liver yielded 40 cc. of thick, greenish yellow pus, which on smear and culture was shown to contain Staph. aureus (poor color producer). November 11, the patient died.

Not only these cultures of the pus but also three of a number of blood cultures revealed Staph. aureus of a type that produced colonies which at first were colorless but which after a week or more became yellowish.

Necropsy.—The body was that of an emaciated, jaundiced adolescent male, with pustules over the face and shoulders and purpura on the legs. The diaphragm, the right lung and the right lobe of the liver were firmly bound together by white pearly fibrous tissue. There was a sinus, 2 cm. in length and 3 mm. in diameter, extending from an abscess in the liver through the diaphragm to the inferior surface of the right lung. The extremely large liver weighed 3,400 Gm. Glisson's capsule was thin and tense. On section the cut edges of the liver became everted. The right lobe contained a large multiloculated cavity, approximately 12 cm. in diameter, with numerous smaller cavities surrounding it (fig. 1). All the cavities were filled with pale greenish white thick fluid and possessed thick fibrous walls and shaggy linings. No granules were visible grossly. The right pleural cavity was obliterated by a pad of thick fibrous tissue several millimeters in thickness. No nodules were seen in the substance of the right lung. In the lower lobe of the left lung there were two widely separated, discrete reddish nodules, firm and noncreptitant, the larger measuring 2 cm. in its greatest diameter. The large, swollen reddish brown kidneys together weighed 650 Gm. Their capsules stripped with ease to reveal smooth red-brown surfaces with numerous punctate red flecks. On section there was eversion of the cut edges. In the wide brown cortices were numerous petechiae. Many bright red striations were evident in the pyramids. The pelves and ureters appeared normal. The remainder of the observations at necropsy were noncontributory.

Microscopic Observations.—Sections of the liver revealed marked separation of the liver cords by edematous fibrous tissue and occasionally the presence of lymphocytes and polymorphonuclear leukocytes. There were coalescing tuberculoid lesions of varying sizes. These had walls consisting of an outer zone of thick collagenous fibrous tissue and an inner one of loose fibrous tissue, capillaries, fibroblasts, epithelioid cells, eosinophils, lymphocytes and rarely foreign body giant cells. In the central areas of liquefaction there was an accumulation of debris, exudate made up largely of polymorphonuclear leukocytes and masses of

Fig. 1.—Actinophytotic abscess of the liver.

coccoid micro-organisms. About each of these masses was a clear zone in the exudate (fig. 2). When stained by the Giemsa method, these masses of micro-organisms had the morphologic character of staphylococci. The bacterial colonies presented a ground substance of amorphous material staining pink with eosin and pale blue with Giemsa stain. About some of these larger masses of organisms there appeared to be refractile capsules. Sections of the sinus tract and of areas of the opalescent right pleural fibrosis showed similar lesions, as did the firm nodules in the lower lobe of the left lung. The collagenous zones about these lesions were thinner than in the liver. The lesion in the kidneys was an acute and sub-acute diffuse glomerulonephritis, as evidenced by the increased cellularity of the

glomerular tufts, many of the cells being polymorphonuclear leukocytes. There was also partial obliteration of many capsular spaces, as well as a number of completely hyalinized glomeruli. There were diffuse interstitial edema and a scattered cellular infiltrate consisting of polymorphonuclear leukocytes, lymphocytes and macrophages.

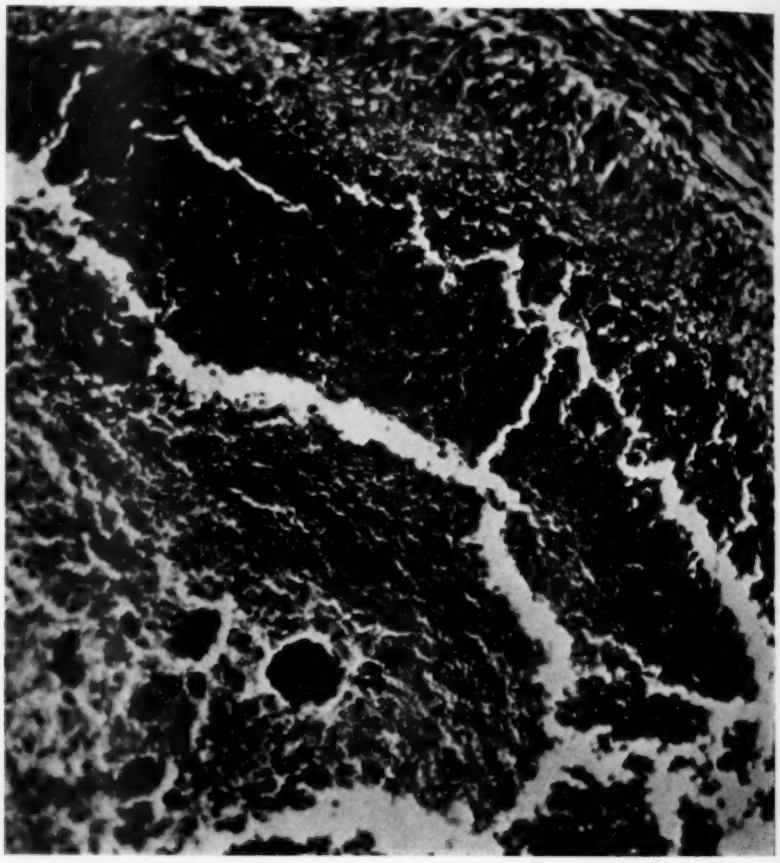

Fig. 2.—Microscopic appearance of the contents of the abscess in the liver; × 400.

Postmortem cultures of the pus from the hepatic abscesses revealed only the Staph. aureus which was a poor color producer. Careful search both in fresh and stained (Pappenheim and acid-fast) preparations failed to reveal any microorganisms other than Staph. aureus.

COMMENT

Staphylococcic actinophytosis has been described here as occurring in the liver and lung of a human subject. This condition occurs occasionally in horses, more infrequently in cattle and swine. In horses it

has been called the *champignon de castration,* since here the lesion has been due to crude methods of castration, after which there has occurred a progressive nodular lesion of the stump of the vas deferens, with later involvement of the internal viscera.

In reviewing the literature, only 8 cases of the lesion in man have been found recorded. Berger [2] and his co-workers collected 4 such cases and reported an additional case, bringing the total to 5. Plaut [3] added 1 case, and 2 more were described by Kimmelstiel.[4] In none of these 8 cases was there involvement of solid internal organs. The first 4 were cases of osteomyelitis; in Berger's case the external genitalia were involved, and in Plaut's, the abdominal wall; in Kimmelstiel's cases there were lesions of the transverse colon and an intraperitoneal mass in the right lower quadrant of the abdomen.

Magrou,[5] in 1914, reproduced the lesion in guinea pigs by inserting into the testicles hairs which had been immersed in cultures of Staph. aureus, these hairs being evidently left in place. He then established that the etiologic agent was Staph. aureus and stated the opinion that the lesion was due to a balance between the number of bacteria and the resistance of the host. In each of the earlier cases in man there were factors suggesting that the presence of a foreign body was of pathogenic importance. However, Kimmelstiel and Easley [6] could not reproduce the characteristic reactions either with or without a foreign body until they very ingeniously devised a method of causing incessantly repeated traumatization and repeated inoculation into the wall of the intestine of small numbers of staphylococci. In drawing their conclusions they stated that botryomycosis (staphylococcic actinophytosis) is due to continuous invasion of tissue by staphylococci. In the case described here, the possibility that a foreign body played a role is extremely unlikely, because of the location of the manifestly primary lesion in the liver and because of metastatic lesions of the same character. There is as well the clinical history of repeated cutaneous lesions from which Staph. aureus was often recovered. Also, on three occasions, blood cultures positive for Staph. aureus were obtained. In the inception of staphylococcic actinophytosis in some instances a foreign body has apparently locally disturbed tissue defense and thus perpetuated staphylococcic infection. However, if, as in this case, there is opportunity for repeated reinoculations with Staph. aureus over a long period, a balance of offense and defense may be achieved which will result in the lesion of staphylococcic actinophytosis without the presence of a foreign body.

SUMMARY

A case of staphylococcic actinophytotic abscess of the liver with extension through the right diaphragm and metastases to the left lung is described.

3. Plaut, A.: Arch. Path. **23**:602, 1937.

4. Kimmelstiel, P., and Oden, P. W.: Arch. Path. **27**:313, 1939.

5. Magrou, J. E.: Les grains botryomyocotiques, Thesis, Paris, no. 267, Paris, Laval, 1914.

6. Kimmelstiel, P., and Easley, C. A.: Am. J. Path. **16**:95, 1940.

Laboratory Methods and Technical Notes

A SILVER IMPREGNATION METHOD FOR RETICULUM WHICH MAY BE USED WITH ANY FIXATIVE AND ANY EMBEDDING MEDIUM

JUAN NEGRIN JR., M.D.,* NEW YORK

It is well known that most of the methods used for the impregnation of reticulum with silver have limitations in routine work. Without examining in detail each method in vogue, these drawbacks may be summarized as follows: Many require special fixation or embedding mediums, and others require varying periods of fixation. Therefore it is obvious that in order to impregnate reticulum with silver, numerous methods have to be employed, depending on different circumstances. In this paper, a method is presented which attempts to eliminate the aforementioned inconveniences.

Because sodium cyanide (NaCN) is used in a method for the industrial extraction of silver, it was thought there might be some application of this substance to silver impregnation of tissues.

After experimenting with many combinations of sodium cyanide and silver solutions on tissues fixed in different ways and embedded in various mediums, it has been found that "cyanidization" before impregnation increased the affinity of the tissue for silver so that silver impregnations were obtained in conditions not possible before.

This fact was observed in nerve tissue as regards neuroglia, microglia, oligodendroglia, nerve cells and nerve fibers; in argentaffin granules of cells of different organs and other fine cellular structures; in argentaffin fibers; in Kupffer cells and in endothelial cells.

The directions referable to reticulum fibers are presented herewith:

1. Fixation: Any kind of fixative can be used—solution of formaldehyde U. S. P.—in different percentages (*B* and *C* in figure); chromic solutions, such as Müller's and Zenker's fluids; Bouin's fluid; alcohol; Kaiserling's solution (*A* in figure); solution of formaldehyde-bromide, and others. No special time of fixation is required.

2. Section: Frozen sections or sections of tissue in any embedding medium, such as paraffin, celloidin (a pyroxylin preparation) or gelatin of any thickness, may be used. For photomicrography, thin sections give better pictures.

3. Sensitization: Immerse in a 10 per cent solution of sodium cyanide at 40 to 50 C. for about ten minutes. Frozen sections can be heated with an alcohol

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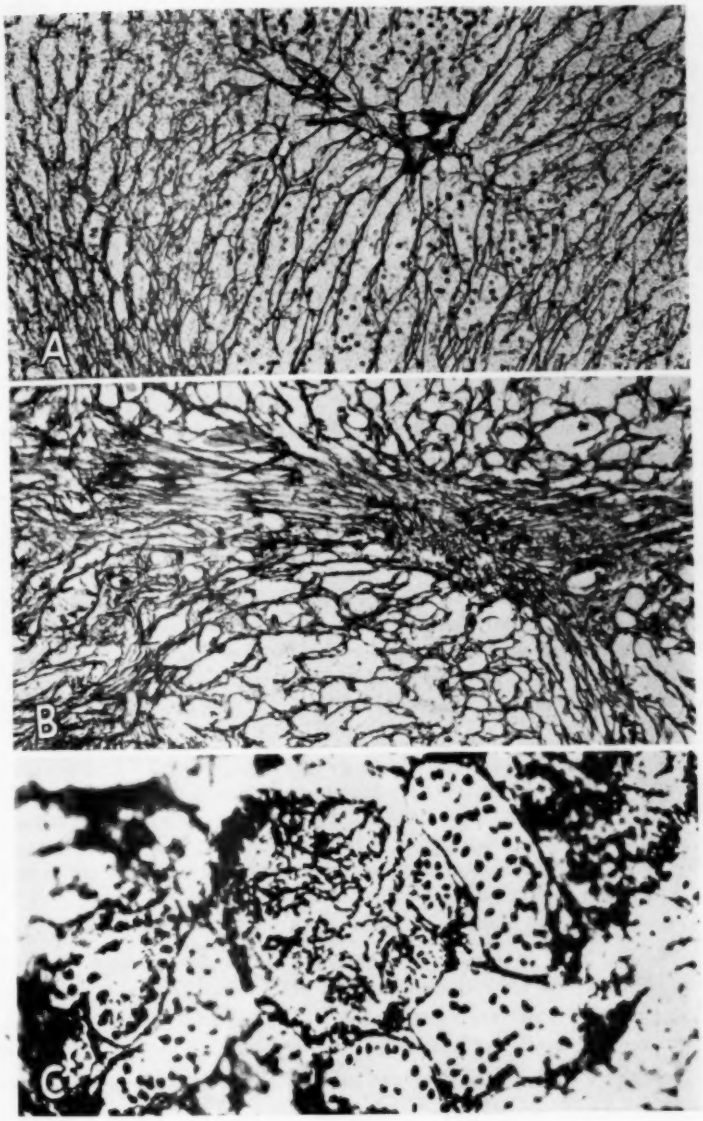

A, reticulum stain in cirrhosis of dog liver. Material fixed in Kaiserling's solution. Frozen section.

B, reticulum stain in cirrhosis of human liver. Material fixed in solution of formaldehyde U. S. P. diluted 1:10. Frozen section.

C, intraglomerular reticulum in human kidney. Material fixed in solution of formaldehyde U. S. P. diluted 1:10. Frozen section.

lamp, and paraffin sections, in an oven. The optimal time for this procedure varies, depending on whether or not frozen sections or paraffin, celloidin or gelatin sections are used. Good results are obtained by heating to 40 to 50 C. for ten to twenty minutes. Sections should not be left in the sodium cyanide solution too long, because they will be destroyed. Paraffin sections tend to fall off the slide, and they should be handled carefully with this in mind. If it is desirable not to impregnate the nuclei, the sections should be left in the sensitizing solution a longer time. If this is done, a white background can be obtained, or nuclear counterstaining can be done if desired. Celloidin sections should not be heated in this solution, because they will be destroyed.

4. Washing: Wash carefully in distilled water two or three times.

5. Impregnation: Place in silver solution for ten to twenty-five minutes at 40 to 50 C., until the sections show a brown color. The silver solution is prepared as follows: To 10 cc. of 10 per cent silver nitrate add ammonia drop by drop until the first formed precipitate is dissolved, being careful not to add an excess of ammonia. In this step Hortega's silver carbonate solution (weak or strong) gives good results. Bielschowsky's silver solution may also be used. Our best results were obtained by using the aforementioned solution. Here the optimum time for silver impregnation depends on the embedding medium and on the fixing solution.

6. Washing: Wash in distilled water containing a little alcohol (to 10 cc. of distilled water add 15 to 20 drops of 95 per cent alcohol). It may be found by others, as it has been by us, that washing with plain distilled water or pyridine water (to 10 cc. of distilled water add 4 to 5 drops of pyridine) is more effective for the impregnation.

7. Reduction: Reduce in solution of formaldehyde U. S. P. diluted 1:100 or a weaker solution (50 cc. of distilled water plus 10 to 15 drops of the 1 per cent dilution of solution of formaldehyde) for one to three minutes. Better results are obtained if neutral solution of formaldehyde is used (solution of formaldehyde U. S. P. or solution of formaldehyde U. S. P. neutralized with magnesium carbonate).

8. Differentiation: Put section in a 10 per cent solution of potassium sulfocyanide and check the optimal time from the first two seconds with a microscope. Generally this differentiation is enough, but sometimes clearer images are obtained if this step follows the fixation (step 11) in sodium hyposulfite. A solution of sodium cyanide may be used, but more care must be taken, since differentiation is very quick and fading of the desired image may occur.

9. Washing: Wash thoroughly in distilled water.

10. Toning: Tone in 1 per cent gold chloride for five minutes at room temperature and then for five minutes at 30 to 40 C.

11. Fixation: Fix for one to five minutes in 5 per cent sodium hyposulfite and then wash thoroughly in distilled water. No washing is required before fixation.

12. Mounting: Dehydrate, clear and mount in Canada balsam. Before mounting, a nuclear counterstain can be done if desired. The result is a white background with or without nuclei. Reticulum fibers are black and other fibers of connective tissue brownish blue.

Distilled water is used for all solutions, as well as for all washing of the sections. All solutions keep for some months. The silver solution can be made up at the moment, mixing 10 cc. of a stock 10 per cent silver nitrate solution in a silver staining dish with ammonia drop by drop until the first formed precipitate

is dissolved. The same 10 per cent silver nitrate solution can be used for months if kept under proper precautions.

With this method, a great deal can be done in connection with the study of reticulum. The finest fibrils can be demonstrated in tissues in which other methods give no good results. The performance does not take more than an hour, which is a relatively short time. Any kind of tissue, fixative, embedding medium or frozen section may be used. Because of these facts, the method is recommended for routine work as well as for special studies.

SUMMARY

A silver impregnation method for reticulum is presented which may be used on any kind of tissue and requires no special fixative or embedding medium. This method is useful in the study of reticulum in routine as well as in research work because of the constancy and homogeneity of the results obtained and the short time required.

General Reviews

THE PATHOLOGY OF BRUNNER'S GLANDS

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Probably there is no part of the human body that has received less attention from pathologic anatomists than Brunner's glands of the duodenum. Morphologic anatomists have studied them extensively, particularly in the field of comparative anatomy, and physiologists have occasionally attempted to solve the secret of their functional status, but the pathologist has rarely given them any special consideration. The reasons for this inattention will be clear when the meager results of the present survey are revealed.

According to Oppel,[1] the glands of the duodenum were first mentioned in 1679, by Wepfer, but nine years later Brunner (or Von Brunn) described them more fully as observed in the duodenums of man, dog, horse and other animals. Accordingly, Middeldorpf,[2] in 1846, called them "Brunner's glands," and since that time the term has obtained almost universal acceptance. It would perhaps be more logical to use the designation "glandulae duodeni" (duodenal glands), as first suggested by Brunner.

ANATOMY

Anatomists are generally agreed that these glands are present in the duodenum in all species of mammals, that they are most abundant immediately distal to the pylorus and that they gradually diminish toward the duodenojejunal junction. This is especially true in man, in whom they are sharply confined to the duodenum, only occasionally failing before, or extending beyond, the beginning of the jejunum. Their chief anatomic peculiarity is their position in the duodenum in relation to the muscularis mucosae. While a small portion of the glands (about a third) are in the deeper layers of the mucosa, the larger bulk are found in the submucosa.

Histologically, they are described[3] as branched tubular alveolar glands lined with large cells having with hematoxylin and eosin a lightly

From the Section on Pathologic Anatomy of the Mayo Clinic.

1. Oppel, A.: Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere, Jena, Gustav Fischer, 1897, pt. 2, pp. 337-375.
2. Middeldorpf, cited by Schwalbe, G.: Arch. f. mikr. Anat. **8**:92, 1872.
3. Tschassownikow, N.: Anat. Anz. **61**:417, 1926.

staining cytoplasm. The cytoplasm gives a strong reaction with Best's carmine stain for glycogen [4] and a faint reaction with the various stains for mucin. Bensley,[5] who has studied these glands extensively, satisfied himself by a special variation of Mayer's mucicarmine technic, that the cells gave a very specific reaction for mucin and that the glands of Brunner "are of the pure mucous type."

Their ducts are lined with cells exactly similar to those of the alveoli, are relatively wide, and empty into the duodenal lumen through the ducts of Lieberkühn's glands. The ducts of the submucosal portions pass through openings in the muscularis mucosae. The marked similarity of the cells of the duodenal glands to those of the pyloric glands in both appearance and staining reactions had led many writers to assume that the former are a direct continuation of the latter. Oppel [6] theorized that phylogenetically duodenal glands arise from extensions of those in the pylorus. The question has aroused considerable controversy and has not yet been definitely decided. However, there is general agreement that in man, as well as in many other animals, the cells of these two gland groups are remarkably alike and that the groups gradually merge, the one into the other. The chief difference is the fact that Brunner's glands break through the muscularis mucosae, whereas the pyloric glands are wholly confined to the deeper layers of the mucosa. Bensley concluded that "certainly they are primitively cells of the same type."

PHYSIOLOGY

Studies of the function of the duodenal glands have given results somewhat contradictory and on the whole disappointing. Brunner concluded that these glands constituted a secondary pancreas, a view which has never received any confirmatory evidence. However, the fact that in the duodenal glands of the rabbit there are "dark cells" similar to those of the pancreatic acini has led to extended discussions and much controversy.

Heidenhain [7] stated that Hirt found in Brunner's glands during digestion changes similar to those seen in pyloric glands. During periods of hunger the cells were relatively large and clear, and during digestion they were small and cloudy. Other workers (Bogomoletz;[8] Helly;[9] Middeldorpf;[2] Grützner,[10] and others) from time to time have reported

4. Feyrter, F.: Virchows Arch. f. path. Anat. **293**:509, 1934.

5. Bensley, R. R.: The Structure of the Glands of Brunner, in Decennial Publications of the University of Chicago, Chicago, University of Chicago Press, 1903, vol. 10, p. 45.

6. Oppel, A.: Ergebn. d. Anat. u. Entwcklngsgesch. **14**:100, 1904.

7. Heidenhain, R.: Arch. f. mikr. Anat. **8**:279, 1872.

8. Bogomoletz, A. A.: Arch. f. mikr. Anat. **61**:656, 1903.

9. Helly, K.: Anat. Anz. **22**:418, 1903.

10. Grützner, P.: Arch. f. d. ges. Physiol. **12**:285, 1872.

the presence of diastatic and pepsin-like ferments, but Hoppe-Seyler [11] emphasized the difficulty of separating the cells and secretions of Brunner's glands from those present in the overlying epithelium and in the lumen of the duodenum. Bensley, by microchemical tests, found no traces of phosphorus and only faint traces of iron in the duodenal glands. In this respect they react like the mucous cells of the salivary glands. He stated that when zymogen granules are present in secreting cells, as in those of the pancreas and stomach, these cells give positive reactions for phosphorus and iron. He concluded, therefore, that Brunner's glands secrete mucous and possibly a very small amount of some unknown ferment.

In more recent years Florey and Harding [12] reported experiments on juices carefully collected from isolated duodenal segments. The fluid thus obtained was thick, viscous and sticky, had the appearance of egg white and contained considerable bicarbonate. They could not be certain whether the bicarbonate was secreted by the cells of Lieberkühn's crypts or was a specific derivative of Brunner's glands but they presumed it came from both groups of cells. From a fistula of a 5 cm. duodenal loop in a goat they collected 96 cc. in twenty-four hours. The dextrose content varied from 13 to 23.2 per cent. The physical character of the fluid suggested the presence of mucin. The giving of food stimulated the secretion, as did also the passing of hydrochloric acid over the duodenal mucosa. Coarse tests failed to demonstrate either amylase or lipase, but in the secretions from the goat and dog proteolytic enzymes active in an acid medium were present. After administration of pilocarpine hydrochloride, both the pyloric and the duodenal glands revealed almost complete evacuation of mucin. The authors expressed the belief that the normal secretion of Brunner's glands, by virtue of its mucin and alkaline content, protects the duodenal mucosa from damage by the acid gastric juice and inferred that malfunction of these glands might be primarily responsible for duodenal ulcer.

In other studies [13] they found that neither the sight nor the smell of food increased the secretion and that histidine had no effect. Atropine did not inhibit the flow, but the intravenous injection of crude or highly purified secretin caused a definite response. They concluded that normally the activity of Brunner's glands is controlled by the presence or the absence of secretin.

Recently Meulengracht,[14] working on the stomachs of pigs, concluded that the specific antianemic factor (intrinsic factor of Castle) is con-

11. Hoppe-Seyler, cited by Oppel,[1] p. 352.

12. Florey, H. W., and Harding, H. E.: J. Path. & Bact. **37**:431, 1933; Proc. Roy. Soc., London, s.B **117**:68, 1935; Quart. J. Exper. Physiol. **25**:329, 1935.

13. Florey, H. W., and Harding, H. E.: J. Path. & Bact. **39**:255, 1934.

14. Meulengracht, E.: Ztschr. f. klin. Med. **130**:468, 1936.

tained in what he called "the pyloric gland organ," consisting of the cardiac, pyloric and Brunner's glands. However, in a study of these glands in several cases of human pernicious anemia [15] he could discover no appreciable change and was forced to suggest that the defective action might be functional and not referable to anatomic alteration.

All in all, one must agree with Tigerstedt,[16] who concluded that "ueber die Sekretionsbedingungen der Brunnerschen Drüsen wissen wir fast gar nichts" (concerning the conditions governing the secretion from Brunner's glands we know practically nothing).

PATHOLOGY

The feeling of frustration which results from an attempt to fathom the function of Brunner's glands is experienced in a more advanced degree when one surveys the literature describing the effects of disease on these glands. In the standard textbooks of pathology the glands are hardly mentioned, and after a fairly extensive search I have been unable to find any report of a systematic or comprehensive study of their changes in disease. In most respects they seem to have almost completely escaped any recorded observations. Apparently they are the forgotten glands of the human body.

One exception in this barren field is the subject of the relation of Brunner's glands to duodenal ulcers. In 1910 Helmholz [17] described changes in the epithelial cells of these glands in the region of active ulcers. The nuclei and cells assumed a resemblance to those of the surface epithelium. He regarded the change as a reparative process, the proliferation being responsible for the initial covering of the ulcer.

Holzweissig [18] studied the scars of duodenal ulcers and implied that in cases of healing ulcer Brunner's glands are not completely restored.

Crohn, Weiskopf and Aschner [19] concluded: "The Brunner's glands were seen on either side [of duodenal ulcers] but they seemed to play no part in the process of repair, having apparently been destroyed at the site of the ulcer during its active stage." Puhl [20] stated that in healed duodenal ulcers "the layer of Brunner's glands can completely fail, or only remnants are to be seen." He could not decide whether the nests of glands remaining in a scar represent a "regeneration" or a residuum. The evidences of regeneration of Brunner's glands did not seem to be

15. Meulengracht, E.: Am. J. M. Sc. **197**:201, 1939.

16. Tigerstedt, R.: Lehrbuch der Physiologie des Menschen, ed. 9, Leipzig, S. Hirzel, 1919, vol. 1.

17. Helmholz, H. J.: Virchows Arch. f. path. Anat. **201**:243, 1910.

18. Holzweissig, M.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **35**:16, 1922.

19. Crohn, B. B.; Weiskopf, S., and Aschner, P. W.: Arch. Int. Med. **35**:405, 1925.

20. Puhl, H.: Virchows Arch. f. path. Anat. **265**:160, 1927.

clear. Finally Florey and Harding,[21] in an experimental study of the healing of artificial defects in the duodenal mucosa, concluded that Brunner's glands are restored, completely regenerating pari passu with the cells of the villi. However, they added: "Nearly all observers are agreed Brunner's glands do not reform except to a small extent beneath the ordinary intestinal mucosa."

A further group of occasional reports is concerned with the question of neoplastic hyperplasia in the duodenum, and here one is faced with confusion worse than confounded. The writings on carcinoma of the duodenum constitute one of the most difficult problems which the careful analyst has to face.

The head of the pancreas, the papilla of Vater, the bile ducts, the gallbladder, the minor papilla and its ducts, aberrant masses of pancreas, gastric mucosa or other glands, the mucosa of the duodenum, Brunner's glands and metastatic nodules from distant tumors are all sources, potential or actual, of cancers of the duodenum. No wonder it usually happens that the pathologist has not given sufficient study to identify with certainty the origin of the tumor, or the process is so far advanced that accurate classification becomes impossible. Particularly difficult is the identification of a cancer as one which arose from abnormal proliferation of the cells of Brunner's glands. No specific anatomic characters for such a tumor have as yet been established.

A good example of this problem is furnished in the case reported by Ewald [22] in 1886. He described a small ulcerated carcinomatous nodule in the duodenum of a woman aged 67 years. He concluded it arose in the scar of a healed duodenal ulcer. Brunner's glands were not mentioned, and no hint was given as to the type of cell from which the growth might have sprung. The following year Orth [23] suggested Brunner's glands as a possible source of primary cancer of the duodenum. In 1894 Olivier [24] reported a small nut-sized tumor above the papilla of Vater that apparently belonged to the pancreas. However, the carcinomatous glands were in close relation to Brunner's glands. In the same year Pic [25] reported that in a woman aged 53 years who revealed a carcinomatous mass in the head of the pancreas the first portion of the duodenum was hard and ulcerated and contained an annular neoplasm which invaded neighboring organs. He concluded that it was a primary carcinoma of the glands of Brunner. The specimen described by

21. Florey, H. W., and Harding, H. E.: J. Path. & Bact. **40**:211, 1934.

22. Ewald, C. A.: Berl. klin. Wchnschr. **23**:527, 1886.

23. Orth, J.: Lehrbuch der speciellen pathologischen Anatomie, Berlin, A. Hirschwald, 1887, vol. 1, pt. 3.

24. Olivier, cited by Staemmler.[33]

25. Pic, A.: Rev. de méd., Paris **14**:1081, 1894.

Letulle [26] in 1907 was termed *metamorphose cancereuse des glandes Brunneriennes du duodenum* (a cancerous change of Brunner's glands of the duodenum). It was a small tumor beneath intact Lieberkühn's glands and contained evidences of neoplastic hyperplasia. However, he stated that the changes were such as are seen in carcinoma from Lieberkühn's glands. Besse,[27] in 1904, described several nodules in a duodenum, just below the pylorus. He did not intimate that they were malignant or that they were derived from Brunner's glands, but his title, *Polyadénome duodénal,* is often mentioned in reviews.

Perhaps the most elaborate attempt to record tumors of Brunner's glands is that of Scagliosi,[28] who, in 1913, gave details of 13 cases in which he found adenoma of the duodenal glands, undergoing carcinomatous changes in some cases, and also nodules of accessory pancreas. His chief interest in both these growths evidently was not so much in their constitution or occurrence as in their supposed relation to duodenal ulcers. He attempted to prove that these tumors, whose neoplastic characters, he admitted, may be exceedingly uncertain, bulge upward beneath the duodenal mucosa, interfere with the secretion of protective mucus and eventually cause necrosis of the surface epithelium. The acids and digestive juices then attack the underlying tissues, even the cancers, and produce the classic duodenal or peptic ulcers. He inferred that duodenal ulcers are caused by hyperplasia of Brunner's glands and that nodules of misplaced pancreas may produce the same result.

In 1915 Bland-Sutton [29] declared that "there is no evidence to support this theory [that cancer may arise in islands of pancreatic tissue] nor that which attributes duodenal cancer to morbid changes in Brunner's glands. . . . Such theories indicate that pathologists, like poets, are not devoid of imagination." In the same year Beckman [30] wrote that "primary cancer in the duodenum probably does not occur" and when present "it arises in the papilla of Vater." Against this view Anschütz and Konjetzny [31] concluded in 1920: "In ulcers which occur in the vicinity of Brunner's glands one often sees atypical growth of the latter which can lead to a cancerous development." They described an operative specimen, a pylorus from a woman aged 42 years, who had had symptoms of ulcer since she was a young girl. Directly on the pylorus was the scar of an old ulcer, and beneath this was an adenomatous

26. Letulle, M.: Compt. rend. Soc. de biol. **62**:859, 1907.

27. Besse, P. M.: Arch. de méd. expér. et d'anat. path. **16**:388, 1904.

28. Scagliosi, G.: Virchows Arch. f. path. Anat. **214**:220, 1913.

29. Bland-Sutton, J.: Tr. M. Soc. London **38**:1, 1915.

30. Beckman, E. H.: The Differential Diagnosis of Lesions of the Stomach and Duodenum, in Collected Papers of the Mayo Clinic, Philadelphia, W. B. Saunders Company, 1915, vol. 7, p. 194.

31. Anschütz and Konjetzny: Deutsche Ztschr. f. Chir. **154**:1, 1920.

growth of Brunner's glands which was plainly carcinoma. They concluded this was an example of an ulcer scar with cancerous degeneration arising from an atypical growth of Brunner's glands. In 1921 Ramond, Vincent and Clément [32] observed in the duodenum of a woman aged 43 years a tumor the size of an egg, 2 cm. from the pylorus. They concluded it was an atypical carcinoma arising from Brunner's glands.

In 1924 Staemmler [33] published a short review of duodenal carcinoma. He appears to have accepted the neoplasms described by Letulle and Pic as arising from Brunner's glands. He agreed with Geiser [34] and the view, which he said was expressed by Henlin,[35] that the hypothesis that cancer may be derived from the duodenal glands has not yet been proved.

Orator [36] gave particular attention to cancers arising from duodenal ulcers and discussed the reported cases. He believed such cancers, which, he stated, are very rare, may theoretically have three sources: (1) Lieberkühn's crypts, as proposed by Waldeyer and Virchow; (2) Brunner's glands, as suggested by Orth, and (3) pancreatic rests (Cohnheim). He concluded that only the first two origins were admissible for cancers arising from duodenal ulcers. He accepted the tumors reported by Ewald [22] and Anschütz and Konjetzny [31] and added one of his own, which he thought probably arose from gastric mucosa drawn downward by the ulcer scars on the pylorus.

Willis and Lasersohn [37] described in a man aged 65 years a polypoid pedunculated tumor of the duodenum 1 cm. distal to the pylorus, which they diagnosed as adenoma of Brunner's glands. Balfour and Henderson [38] also reported a case of adenoma of these glands.

Oberndorfer,[39] in reviewing the tumors of the intestine, reported a case of adenoma of Brunner's glands, the illustration of which almost exactly matches the picture in one of my own cases. The tumor was about the size of a cherry. He mentioned the cases reported by Olivier,[24] Scagliosi [28] and Anschütz and Konjetzny [31] but added that only a few of these will bear criticism. He accepted the case reported by Anschütz and Konjetzny, although there is still a question of gastric carcinoma appearing in the duodenum. He concluded that in some cases the cancers

32. Ramond, F.; Vincent and Clément, R.: Bull. et mém. Soc. méd. d. hôp. de Paris **45**:1260, 1921.

33. Staemmler, M.: Die Neubildungen des Darms, in von Bruns, P.: Neue deutsche Chirurgie, Stuttgart, Ferdinand Enke, 1924, vol. 33-a, pt. 1, p. 61.

34. Geiser, J. F.: Deutsche Ztschr. f. Chir. **86**:41, 1907.

35. Henlin, cited by Staemmler.[33]

36. Orator, V.: Arch. f. klin. Chir. **134**:736, 1925.

37. Willis, A. M., and Lasersohn, M.: Ann. Surg. **82**:952, 1925.

38. Balfour, D. C., and Henderson, E. F.: Ann. Surg. **89**:30, 1929.

39. Oberndorfer, S.: Die Geschwülste des Darmes, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 4, pt. 3, p. 717.

supposed to arise on the basis of duodenal ulcers or from Brunner's glands really spring from heterotopic bits of gastric mucosa. This is true, he believed, of Orator's and also Ewald's cases.

In 1932 Rutishauser [40] exhaustively reviewed cases of carcinoma of the duodenum. In 1 of his own 6 cases, in which the patient was a man aged 50 years, he concluded that the growths arose from Brunner's glands. There were three nodules in the upper portion of the duodenum which proved to be cysts filled with mucin. Other nodules were in the liver and peripancreatic lymph nodes and on the peritoneal surfaces. Since he described the gallbladder as adherent to the duodenum, his diagnosis of primary multiple malignant adenoma of the duodenal glands is open to rather serious question. The gallbladder, bile ducts and pancreatic ducts must all be excluded as more probably primary sites. He did not accept Letulle's case but mentioned without comment the one reported by Ewald. He said that Besse's case was the only one in which multiple adenoma of Brunner's glands was found. He noted that a cancer in the subpyloric region may arise from Lieberkühn's gland or, as Aschoff pointed out, from misplaced gastric mucosa. He doubted the conclusions of Geiser in respect to carcinoma supposedly arising on the margin of duodenal ulcer.

Feyrter, in 1934, published the results of a careful study of the duodenums of 2,800 persons and classified the observed hyperplasia into three types: 1. "Hyperplasia diffusa et nodosa glandularum duodeni." Coarse folds and irregularly circumscribed nodules made up of multilobulated masses of Brunner's glands were located throughout the greater portion of the duodenum. He mentioned the fact that these cells in part gave a positive reaction with Best's carmine stain and occasionally contained droplets of doubly refractive lipoids. Because of the presence of lymphocytes, plasma cells, occasional eosinophilic leukocytes and rarely mast cells, as well as Russell's bodies, in the mucosa, he concluded that this type of hyperplasia was due to a low grade inflammation. He observed this type in 14 persons, only once in a woman and in none before the age of 24 years. He noted how frequently such hyperplasia is found in cases of severe renal damage and theorized as to the possible influence of urates and other retained substances in causing overgrowth of Brunner's glands. He thought Besse's case might belong in this group. 2. "Hyperplasia nodularis circumscripta glandularum duodeni." In this type, isolated nodules of Brunner's glands were present, especially in the superior portions of the duodenum. There usually was atrophy of the remaining glands. This type was seen twenty times, in 11 men and 9 women, and except for 2 the patients were more than 21 years of age. Uremia or severe renal disease was present in 11 of the patients. He thought some injury must have preceded the hyper-

40. Rutishauser, E.: Frankfurt. Ztschr. f. Path. **43**:133, 1932.

plasia, although heredity might also have had an influence. 3. "Adenoma glandularum duodeni." Tumor-like growths were found in 3 persons. These were single nodules, varying in size from that of a pea to that of a hazelnut. They represented the extremes of nodular hyperplasia. One had cystic dilatations, contained degenerated cells and masses of mucin and presented a granulomatous appearance with collections of lymphocytes. The 3 patients were men more than 54 years of age, and in all 3 the remaining glands were atrophic.

Feyrter cited a few cases from the literature, stating that the earliest was reported in 1876, by Salvioli,[41] and accepted those reported by Scagliosi and Oberndorfer. He agreed with the assertions by Anschütz and Konjetzny and Orator that there is hyperplasia of the duodenal glands at the margins of ulcers and said he had observed this proliferation in the scars of ulcers in 7 persons, 3 with severe renal disease. However, he thought, with Staemmler and Orator, that the Scagliosi hypothesis was not well founded.

In 1937 Stewart and Lieber [42] reported studies of carcinoma of the suprapapillary portion of the duodenum. They abstracted from the literature 104 cases, 35 of which they regarded as acceptable. They admitted the possibility that Brunner's glands were the source of the cancers in 3 cases but regarded the evidence as doubtful. They concluded that there is no proved case of carcinoma arising from Brunner's glands or from a duodenal ulcer.

PRESENT STUDY

My attention was first attracted to Brunner's glands by some detailed investigations of the pathologic anatomy of duodenal and gastric ulcers.

Among others, there are two outstanding differences between these two lesions, whether active or healed. Most gastric ulcers are comparatively shallow and heal without a grossly demonstrable scar, while most duodenal ulcers are deep, even in the acute stage, and leave easily recognizable and oftentimes badly deforming scars. Incidentally, I am convinced that this is one reason why duodenal ulcers are seen at roentgenologic and postmortem examinations so much more frequently than are gastric ulcers. In searching for an explanation of this easily verified observation I was led to examine more closely the duodenal glands underlying the mucosa.

One cannot study many sections of duodenums without understanding quite readily why pathologists have largely disregarded Brunner's glands. Hardly any more stable or colorless set of tissues can be found in the body. Not only are they not subject, so far as is known, to any

41. Salvioli, cited by Feyrter.[4]

42. Stewart, H. L., and Lieber, M. M.: Arch. Surg. **35**:99, 1937.

special reactions to disease processes but, what is infinitely more important, neither their lack nor their excess produces any definite effects on the bodily economy during life. In short, there is not as yet any disease known as hyperadenosis or hypoadenosis of Brunner's glands, and in no medical dictionary can be found the term "acute (or chronic) Brunner's adenitis."

Nevertheless, these specialized glands of the duodenum, which have such striking anatomic characters, are subject to definite departures from the normal, and these I propose to discuss briefly in the order and under the headings usually employed in presenting the pathology of any organ or group of tissues. These observations have been made

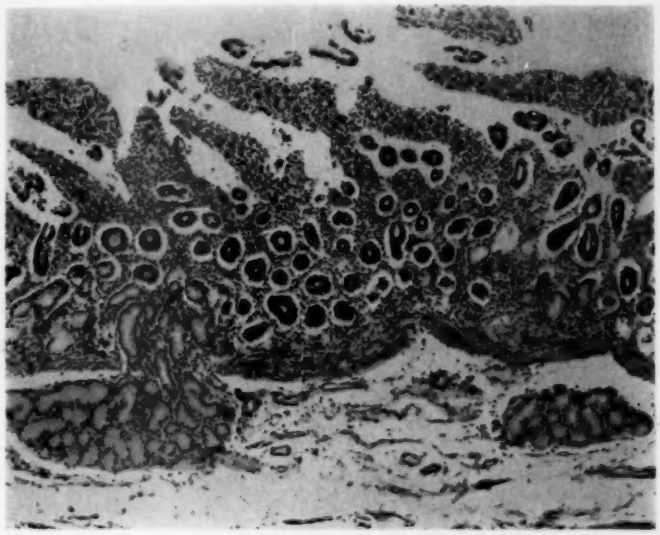

Fig. 1.—Atrophic Brunner's glands (hematoxylin and eosin; × 55). No ulcer is present.

during gross examination of the duodenum in more than 15,000 cases and microscopic study of portions in more than 1,000 of these cases.

Congenital Malformation.—Complete absence of these glands, as far as I am aware, has never been recorded or observed. Also maldevelopment in the form of misplaced portions of the glands or irregularities in the normal differentiation of the cells has not been seen.

Atrophy and Hypertrophy.—The actual quantity of Brunner's glands varies within wide limits, and there is no indication as to the factor or factors which bring about this variation. In some duodenums only a comparatively thin layer of cells lies beneath the muscularis mucosae (fig. 1), and often there are wide spaces without any glands at all. As

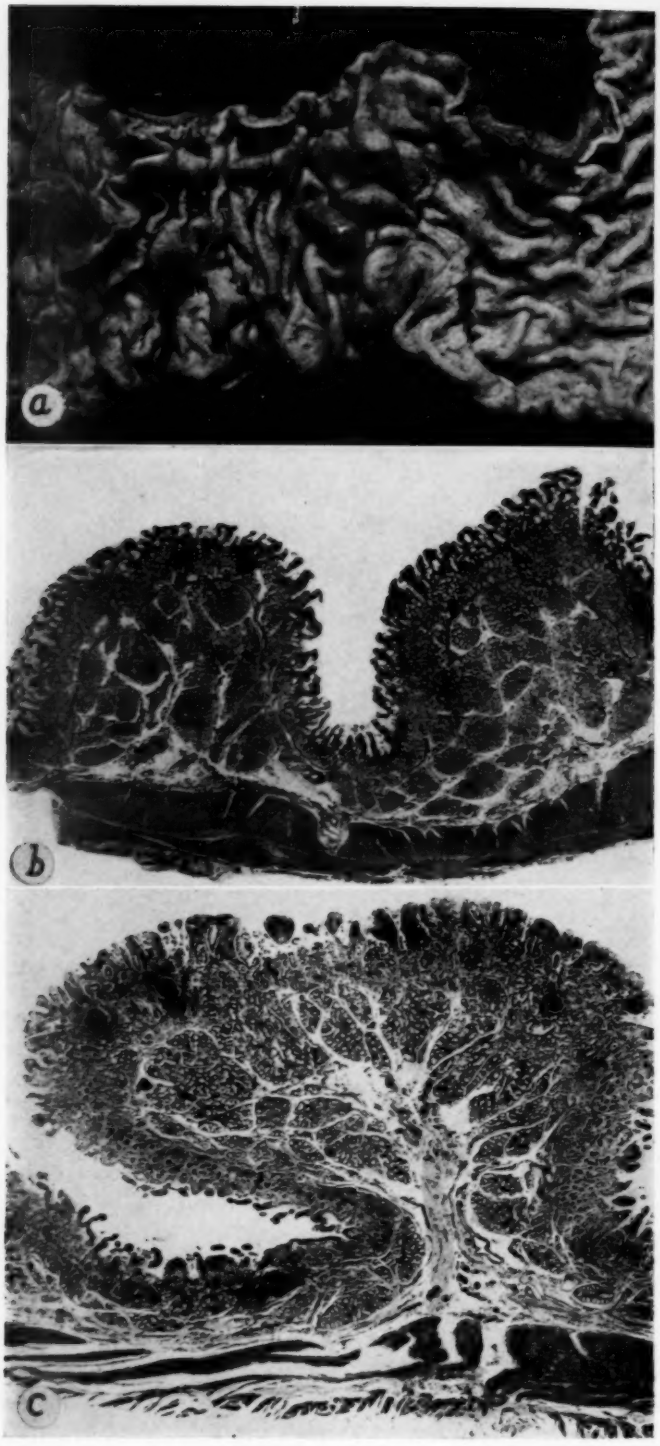

Figure 2

(See legend on opposite page)

is characteristic of the glands under any conditions, they are most thickly segregated in that portion of the duodenum adjacent to the pylorus and rapidly diminish below the papilla of Vater, where they are sometimes not present except for occasional small lobules of cells. While advanced age undoubtedly is accompanied by atrophy of these glands, apparently they are not affected by senility to the same degree as other glandular structures of the body.

Hypertrophy of the duodenal glands causes pronounced thickening of the walls, especially near the pylorus. Not only is there an increased number of lobules but the acini and the cells lining them are larger than normal. The glands may be diffusely and evenly distributed, or they may cause coarse ridges and multiple rounded prominences in the mucosa (fig. 2 *a* and *b*) or, more rarely, solitary fairly large nodules are present, as described by Feyrter. With the latter there may be polypoid formations (fig. 2 *c*) extending into the duodenal lumen, and the neighboring glands may be relatively atrophic. Whatever cause produces this hypertrophy, it does not appear to be related to any neoplastic process. Perhaps heredity or some obscure hormonal influence plays a determining role.

Degeneration and Infiltration.—The outstanding characteristic of the glands is their comparative freedom from influences which ordinarily affect secreting tissues. General toxemias, starvation states and dietary deficiencies apparently leave them unscathed. The cells at times have an almost clear cytoplasmic content, staining only faintly or not at all with ordinary dyes or even with specific mucin stains, and the ducts are free from stainable substances. This condition has been pointed out as evidence of increased function, but I have observed it in the region of duodenal ulcers and other inflammatory processes in the mucosa of the duodenum. Occasionally there is stasis of secretion, with dilated ducts and acini. In some cases this is evidently because of obstruction to the ducts; it has been observed in the region of invading or metastatic tumor nodules and on the margins of chronic or healed ulcers. Such a condition may lead to cyst formation, in which acini or even an entire lobule may be dilated and contain a mass of cell detritus mixed with varying amounts of mucin (fig. 3). More rarely occur large cysts reaching several millimeters in diameter and producing easily observable nodules

EXPLANATION OF FIGURE 2

(*a*) Hyperplasia of Brunner's glands in the subpyloric region. (*b*) Hypertrophy of Brunner's glands (hematoxylin and eosin; × 9). (*c*) Hypertrophy of Brunner's glands with pseudopolyp (hematoxylin and eosin; × 12).

in the duodenal wall (fig. 4 *a* and *b*). These cysts are lined with low cuboid cells, and the lumens are largely free from stainable substances.

Of infiltrations there is little to record. In severe general amyloidosis, the amyloid substance may occasionally be found surrounding atrophic acini. Blood pigments, i. e., hemosiderin, are almost never observed, perhaps because of the rarity of hemorrhages. In cases of hemochromatosis, some iron is deposited in the cells of the glands. Calcium is almost never found, and lipoids, including cholesterol, are never prominent. The significance of the fact that Best's carmine stain for glycogen gives a positive reaction in the cytoplasm of the cells of

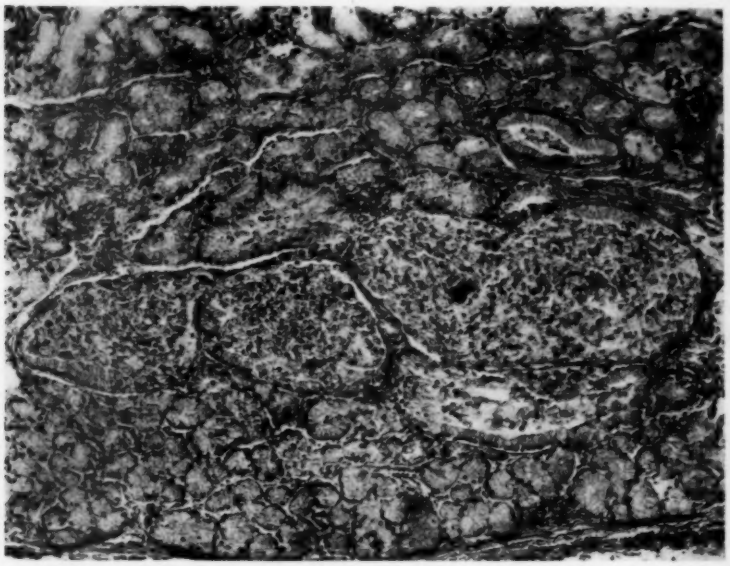

Fig. 3.—Cystic degenerating Brunner's glands (hematoxylin and eosin; ×75).

Brunner's glands is not known. The finding probably represents a reaction with the mucinous compounds which the cells undoubtedly contain and which are some form of glycoproteid.

Foci of lymphocytes are exceedingly frequent in the mucosa and submucosa of the duodenum; they are more prominently present in the later years of life. Usually described as infiltrations, they are more properly to be regarded as the local accumulation, or better, proliferation, of lymphocytes. Their actual meaning is just as obscure as is the meaning of lymphocytes anywhere else in the body. To talk glibly of chronic inflammation may advance one a step but adds little toward clearing the mystery that in general surrounds all lymphocytes.

Inflammation.—In the presence of acute inflammatory conditions in the mucosa of the duodenum, even with erosions or penetrating ulcers, Brunner's glands are apparently noncombatants. Only once have I seen

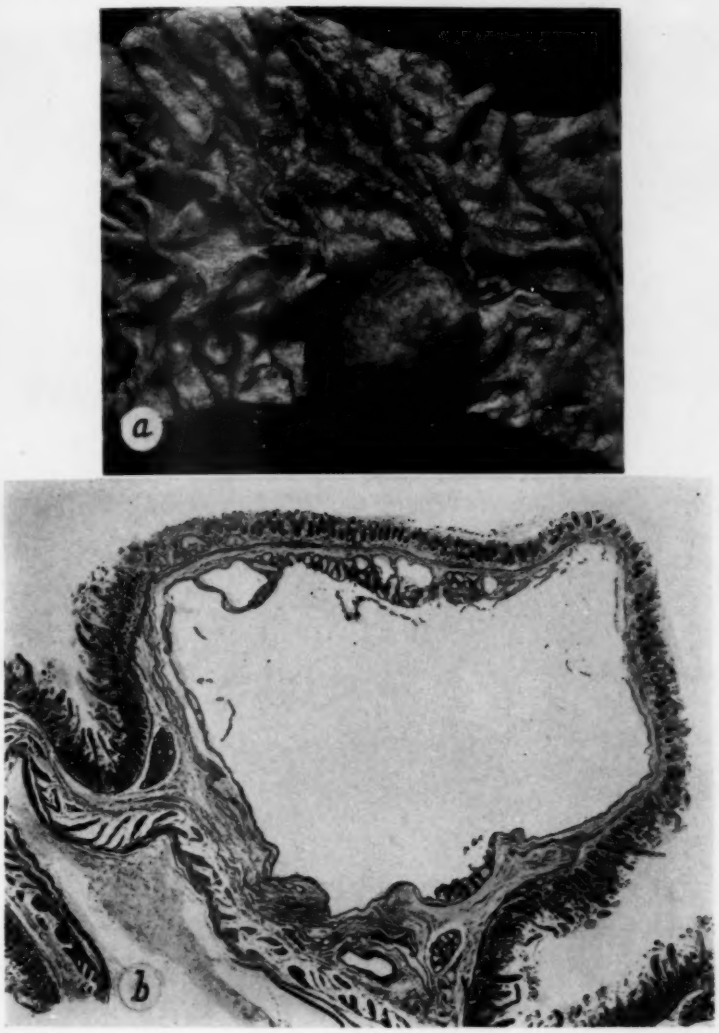

Fig. 4.—(*a*) Cyst of Brunner's glands (near minor papilla). (*b*) Cyst of Brunner's glands (hematoxylin and eosin; × 14).

polymorphonuclear leukocytes in the lumen of a gland duct. Some of the lobules have dilated acini, and as the inflammatory process invades the submucosa, the glands in the immediate region slowly disintegrate without producing much detritus or attracting leukocytes or leaving

other demonstrable residuum (fig. 5 *a*). The glands become *spurlos versenkt*. As the ulcers heal and fibrosis ensues, either the glands are entirely absent (fig. 5 *b*) or islands of small lobules persist (fig. 5 *c*).

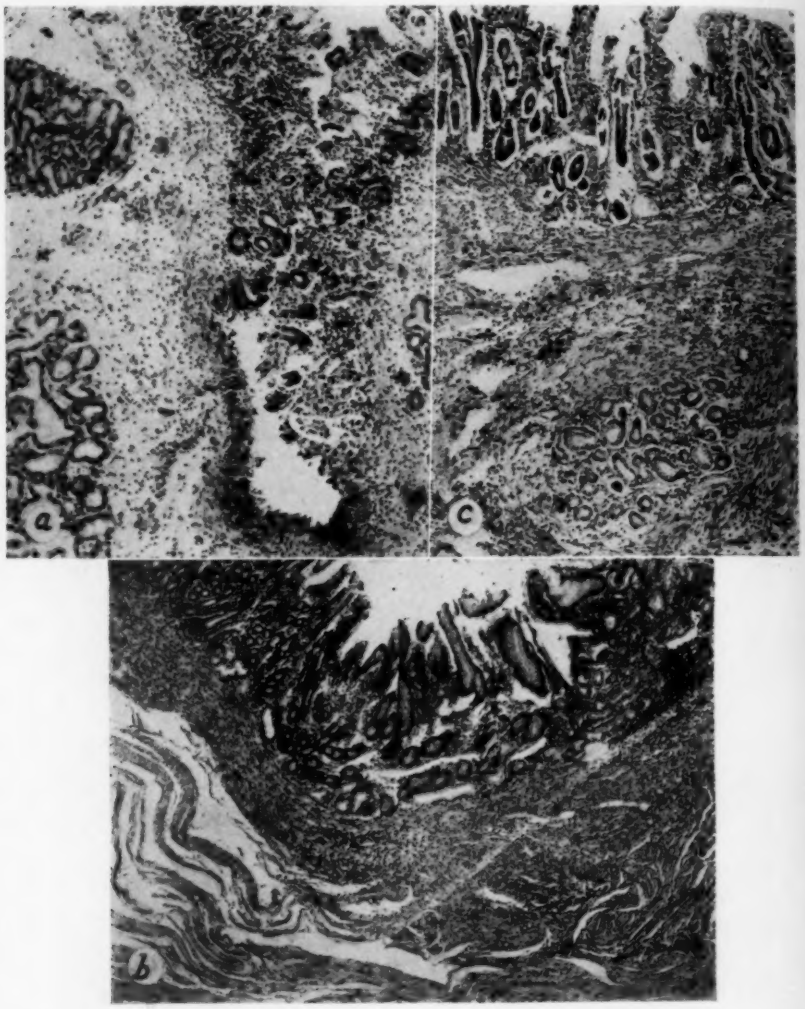

Fig. 5.—(*a*) Deep ulcer cleft with dilated and disappearing duodenal glands (hematoxylin and eosin; × 59.5). (*b*) Healed ulcer, with disappearance of glands (hematoxylin and eosin; × 32.5). (*c*) Scar of ulcer. Note isolated gland lobule (hematoxylin and eosin; × 49.5).

This fact probably accounts for the increased depth of duodenal ulcers as compared with gastric ulcers, as well as for the relative prominence

of the scars of the former and the greater distortion of the first portion of the duodenum so often seen in chronic ulcers and scars. With lesser destruction of the glands, interlacing bands of scar tissue appear in and

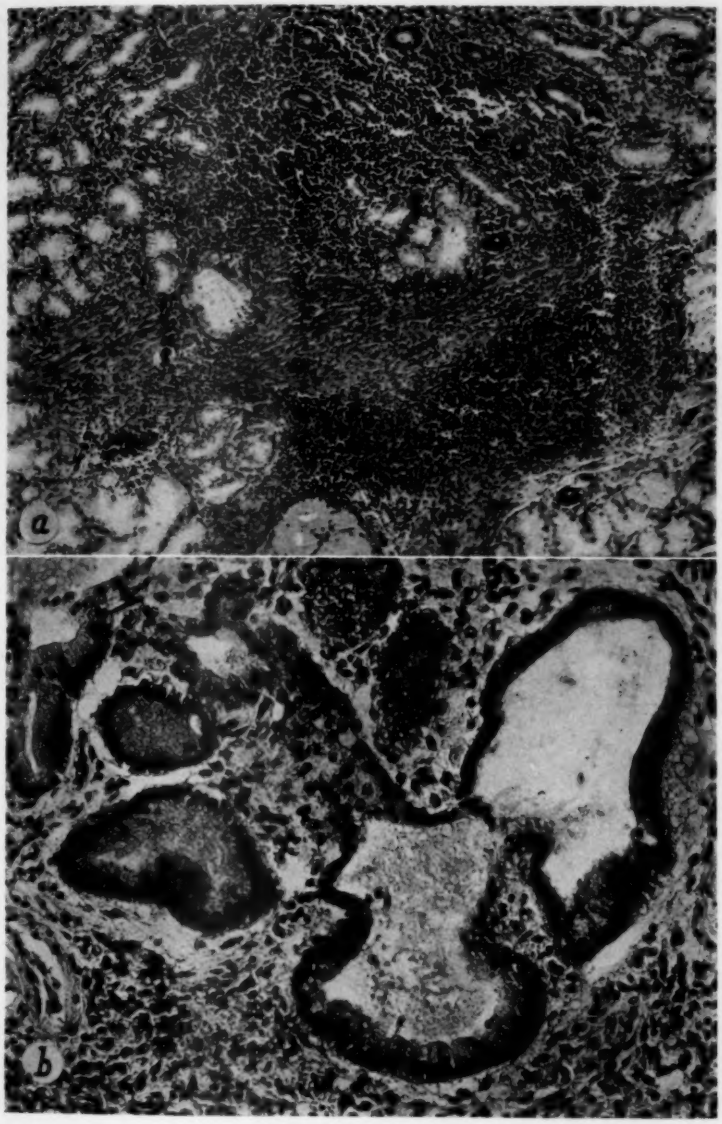

Fig. 6.—(*a*) Focal scar and lymphocytes (hematoxylin and eosin; × 65). (*b*) Chronic ulcer with hyperplasia of the cells of Brunner's glands (hematoxylin and eosin; × 155).

around the gland lobules (fig. 6*a*). In more severe destruction the residual glands and their ducts are often dilated and their mucin content has disappeared. Special inflammatory reactions to acute or chronic

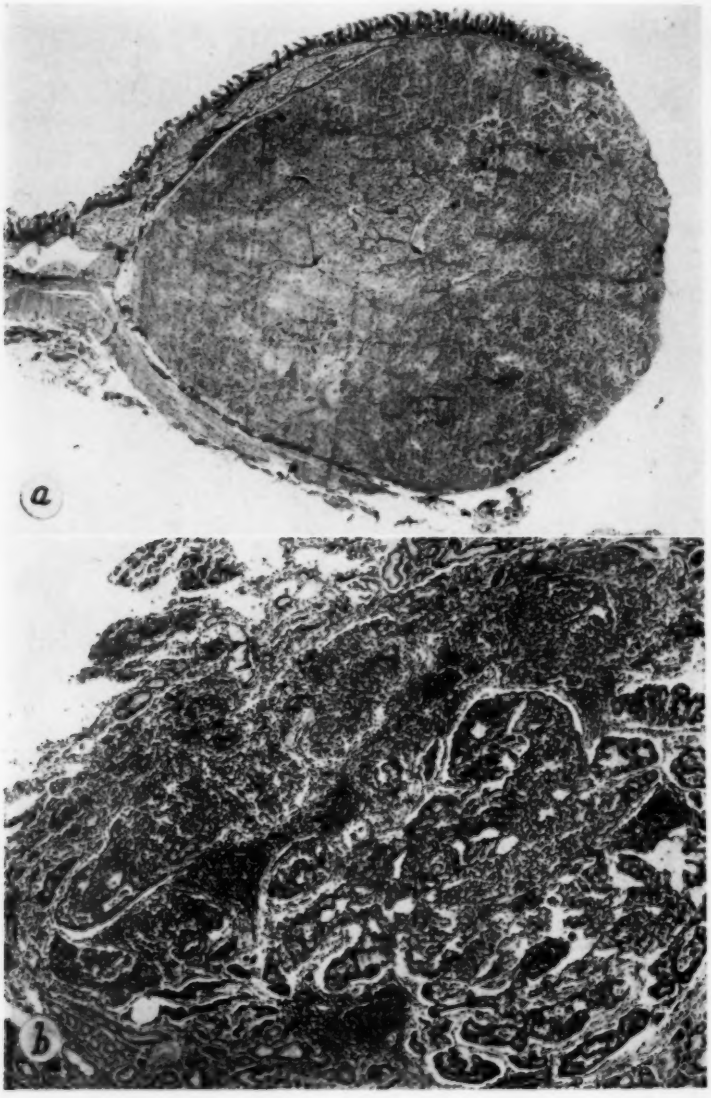

Fig. 7.—(*a*) "Adenoma" of Brunner's glands (hematoxylin and eosin; × 52). (*b*) Beginning carcinoma of Brunner's glands (hematoxylin and eosin; × 50).

granulomatous infections are not described, and I have not observed them. In the presence of the very rare tuberculous ulcers of the

duodenum the glands behave just as they do in the presence of non-specific ulcerations.

Hyperplasia and Neoplasm.—In this category is encountered one of the most important phases of pathologic alteration of any organ or tissue, and here again, as in the presence of other features, Brunner's glands exhibit their usual sluggishness. I have studied many series of glands, especially in the region of duodenal ulcers in all stages of development and healing, and only twice have I observed evidences of proliferative activity. These consisted of hyperchromatism of the nuclei, decrease in the cytoplasm and occasional mitosis (fig. 6 *b*). With the remainder of the ulcers, the glands were either gradually disintegrating or completely demolished.

As to real neoplasms, the literature already indicates their great scarcity. Most of the so-called examples of adenoma (fig. 7 *a*), which I have seen duplicated in the duodenums examined, are more properly to be regarded as examples of hypertrophy due, as previously suggested, to some functional, hormonal, hereditary or unknown influence. In my collection, several growths suspected of representing neoplasia of Brunner's glands failed, after careful review, to indicate with any certainty their origin from these glands. One only has some of the characters of unorganized hyperplasia (fig. 7 *b*). This occurred in a man aged 59 years, who died of decompensated hypertension and was accidentally observed in a routine examination. It was evidently in the early stages of growth, but the cells were hyperchromatic and irregularly arranged and exhibited occasional mitotic figures. Secretion was noticeably absent. Incidentally, it is only in the small early growth that the exact relation of any cancer to its primary source can be determined with accuracy.

COMMENT

Unfortunately, this brief summary of the principal facts regarding Brunner's glands gives little insight into their significance either in health or disease. The reason for this may be that they are apparently, or relatively, really unimportant. As Meulengracht contended, they may have a function closely related to that of the pyloric and cardiac glands of the stomach, which they resemble anatomically to such a degree that many anatomists consider them simply an extension of the pyloric glands. Meulengracht's hypothesis that all three groups furnish a specific antianemic factor needs confirmation. Comparative studies of their behavior under varying conditions are highly desirable. Cursory study has given me the impression that both pyloric and cardiac glands exhibit more active anatomic changes and therefore probably possess more functional capacities. At least hyperplasia and carcinoma are much more in evidence in these glands than in Brunner's glands.

Because of their general appearance, the duodenal glands have often been likened to the salivary glands, and it is true they have several features in common, but knowledge of the function and changes in disease of the latter group is much more exact, and grouping both together does not serve to explain some of the major peculiarities of the duodenal group. Again it may be conjectured that Brunner's glands are vestigial remnants and have no further important role in the bodies of mammals of at least the higher orders. The lack of any definite proof as to their function and their almost uniform passivity under varying normal and pathologic conditions lend support to this conception. Against this hypothesis must be noted that they are consistently present in every species of mammal and that in all of these, especially in man, they are heavily concentrated immediately distal to the pylorus. It is in this area that the acid stomach contents come into contact with the duodenal, pancreatic and hepatic secretions. The secretions of the duodenum, including those of Brunner's glands, are relatively abundant, and while important enzymes may not be secreted by these glands, their alkaline mucus may furnish exactly the necessary elements for protecting the mucosa and preparing the chyme for further digestion and absorption. It is possible also that the succus entericus and enterokinase may finally be proved to have their source in whole or in part in Brunner's glands.

Even if the pathologist can find in Brunner's glands little to interest him, their functional status in the human body would appear to demand more exact and definitive evidence than has yet been adduced.

SUMMARY

Brunner's glands have been known and studied by anatomists for more than two hundred and fifty years. They are acinotubular glands which are present in all species of mammals, roughly mark the limits of the duodenum, are largely located in the submucosa and tend to be concentrated in the proximal portion of that organ. Mucin, glycogen, proteolytic ferments and carbonates are supposed to be present in the secretion of these glands. During digestion there apparently are slight alterations in the appearance of the cells. Pathologically, they are marked by an outstanding passivity and a lack of any special or prominent alterations under abnormal conditions. The glands vary in quantity within wide limits, sometimes exhibiting atrophy and more rarely prominent hypertrophy in diffuse or nodular forms. Dilatation, cyst formation and disintegration are fairly frequent. In the region of chronic and healed duodenal ulcers they are definitely diminished in quantity. Hyperplastic and neoplastic involvements are very rare. Only a few of the reported cases of carcinoma arising from these glands are to be accepted without reservations. Their true functional status deserves more exact study.

Notes and News

Awards.—James Ewing has been awarded the Clement Cleveland Medal of the New York City Cancer Committee for outstanding work in the campaign to control cancer.

M. Pinson Neal, professor of pathology in the University of Missouri, was given the service award for 1940 of the Mississippi Valley Medical Society.

I. Forrest Huddleson, research professor of bacteriology at Michigan State College, was awarded the prize for 1940 of the twelfth International Veterinary Congress. This prize is awarded each year to a member of the American Veterinary Medical Association whose work during the year has been the most outstanding and valuable to the advancement of veterinary science.

The Sedgwick Memorial Medal of the American Public Health Association has been awarded posthumously to Hans Zinsser.

Society News.—The American Association of Pathologists and Bacteriologists will hold its next annual meeting in New York University, April 10 and 11, 1941.

At its sixty-ninth annual meeting, the American Public Health Association elected W. S. Leathers president, John L. Rice president-elect and R. M. Atwater executive secretary. The next meeting will be held in Atlantic City, in October 1941.

New Fellowship.—The Chicago Heart Association has received a gift of $27,000 from the Clara A. Abbott Trust to be added to the Memorial Fund established by Dr. and Mrs. Morris Fishbein in memory of their son who died from rheumatic fever. A fellowship will be devoted to the study of the cause and treatment of rheumatic fever, in a hospital or medical school in Chicago.

Pure Genetic Strains.—The Division of Biology and Agriculture of the National Research Council, Washington, D. C., has set up a committee to promote the maintenance of pure genetic strains of animals and plants needed for investigative purposes in medicine, genetics and related fields.

Book Reviews

Bacillary & Rickettsial Infections—Acute and Chronic. William H. Holmes, professor of medicine, Northwestern University Medical School; chairman, department of medicine, Passavant Memorial Hospital, Chicago. Cloth. Pp. 676. Price $6. New York: The Macmillan Company, 1940.

The author[1] has patterned a medical textbook on a literary rather than a purely technical plan. In the preface he analyzes the strictures of the modern medical curriculum that contribute so potently to the stereotypy of medical texts. His hope for the student in departing from the beaten path is "to sharpen his critical faculties while acquiring habits of thinking and study, and this would result in his becoming a man of wide interests and broad vision and, eventually, perhaps in his becoming an educated physician" (p. 1, "Preface"). From a pedagogic standpoint, any further stress of didactic courses in internal medicine strikes a discordant note. Certainly, the modern movement in medical instruction is strongly away from this plan.

The critical reviewer will revel in the superior construction of this volume. The historical background is evolved without pedantry. In the unfolding of the story only one detraction is encountered. The biographic sketches, while most interesting to the historically minded, tend to disconcert the reader by disturbing the continuity of the story. One of the most fascinating contributions to this text is the inclusion of classical descriptions of the several diseases from original sources, lay and medical. Quite naturally, Aretaeus, Sydenham, Thucydides, Boccaccio and others are herein represented. Unusual attention is given to bacteriology and immunology. Indeed, in certain relations the technical considerations in these fields have been carried to immoderate extremes. The text is replete with classical allusions. Rats and mice multiply—"Not having a Malthus of their own and not having heard of ours" (p. 14). The chapter on undulant fever is thus introduced:

"What imaginative beings our ancestors were! They not only worshipped bull-gods, such as the Thracian Dionysos and the Cretan Minotaur, but they filled the woods with gods and demi-gods, with Satyrs, Pans, Silvanuses and Fauns. The appearance of the entrails of an animal was used by them to foretell events. They believed that by eating the flesh of an animal which they worshipped they would become god-like; that, by drinking its blood, they would obtain absolution from their sins; or that they could transfer their sins to an animal and chase it into the wilderness as a scapegoat."

Dr. Holmes has utilized modern as well as ancient historical sources and some of the most effective are garnered from these sources, as witnesses the following description of a prison camp in the Civil War:

"As these waters, loaded with filth and human excrement, flow sluggishly through the swamp below, filled with trees and reeds coated with a filthy deposit, they emit an intolerable and most sickening stench. Standing as I did over these waters in the middle of a hot day in September, as they rolled sluggishly forth from the stockage, after having received the filth and excrement of twenty thousand men, the stench was disgusting and overpowering; and if it was surpassed in unpleasantness by anything, it was only in the disgusting appearance of the filthy, almost stagnant waters moving slowly between the stumps and roots and fallen trunks of trees and thick branches or reeds, with innumerable long-tailed, large white maggots, swollen peas, and fermenting excrement, and fragments of bread and meat."

The author is meticulous in expression as well as in organization of materials. For example, he refers to the "tubercular" process and the "tuberculous" patient.

1. Dr. William H. Holmes died, after a long illness, Nov. 2, 1940.

Exception may be taken to the designation "unfaithful Anne Boleyn" (p. 62). The English clergyman interested in science was Stephen Hales, not Stephen Hale (p. 64). The American contributor to the knowledge of Rocky Mountain spotted fever was Maxcy not Maxey (pp. 87 and 89). Sanatoria is spelled "sanitoria" (p. 497).

The clinical discussions are, as anticipated, very strong. The detailed discussions of certain technical procedures, as tracheotomy (p. 340), should, in the judgment of the reviewer, have been left to the special fields of their application. This same objection may be entered against the inclusion of the surgery of Pott's disease (pp. 539 to 543). An illustration of the author's effective use of figures of speech is found under the discussion of the treatment of diphtheria (p. 303): "Antitoxin given on the first day of illness is comparable to an army, on the second day to a corps, on the third to a division, on the fourth to a battalion, on the fifth to a squad, and on the sixth to a lone soldier without a gun." In the discussion of the treatment of tetanus an inadvertent contradiction appears. A group of British wounded unprotected by tetanus antitoxin showed a mortality of 53.3 per cent; whereas, enormous doses of antitoxin effected a reduction to 47.3 per cent mortality. These figures do not constitute a reduced mortality rate of 43 per cent as given in the succeeding sentence. Histaminase as a protection against serum sickness is not mentioned; nor is the use of the roentgen ray in the treatment of gas gangrene suggested. The reviewer heartily concurs in the author's conclusion that "to eradicate tuberculosis we must concentrate our efforts to a greater extent than heretofore upon the soil in which it grows" (p. 610).

This book deserves a place in the library of the thoughtful physician. Its clinical approach will not have a great appeal to the busy commercial practitioner interested in monetary returns rather than in the evolution of the knowledge of disease. Nor will the student, heedless of the advantages of the classical approach, use the book as his working source to the exclusion of the utilitarian volumes. Nevertheless this book sets a new high in the field. Its format is interesting and the margination of leading topics helpful. The paper and type are not superior. Objections to the size of the latter may reflect the presbyopia of the reviewer.

Atlas of Cardioroentgenology. Hugo Roesler, M.D., F.A.C.P., associate professor of roentgenology, cardiologist in the department of medicine, Temple University School of Medicine and Hospital, Philadelphia. Cloth. Pp. 124, with 166 illustrations. Price $8.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1940.

This work may be regarded as a continuation of the author's "Clinical Roentgenology of the Cardiovascular System," published in 1937. It is a worthy successor to that volume. It should interest and stimulate clinicians, pathologists and roentgenologists. Perhaps the most important lesson inculcated is that more and more is it becoming evident in these days of specialism that an understanding of disease, especially in the way of accurate diagnosis, is a matter of team work. In the case of heart disease, for example, dependence cannot be placed alone on history, physical examination, laboratory tests, electrocardiogram or roentgenogram. Each one of these has its place, but each has contacts with, and is mutually dependent on, all the others. And, besides, no one of these tests is properly utilized unless it is based on an understanding of the anatomy and function of the parts involved together with a clear notion of the pathologic changes that may alter anatomic relations and disturb function. These truths are repeatedly called to mind by the condensed case reports that are presented, with the symptoms, the temperature and pulse records, blood counts, blood pressure readings and blood composition, and the electrocardiograms and roentgen films taken in various positions. Often the most instructive feature is the revelation of the autopsy that explains the symptom not well understood during life or makes clear why the roentgen silhouette was so puzzlingly distorted. The stress in this atlas, naturally, is on the roentgen features. Dilated aortas, parietal cardiac aneurysms due to old infarctions, cardiac changes due to valvular lesions of various types are all clearly

shown. The accuracy with which sclerosed valves and sclerotic coronary arteries can in some cases be made out in the roentgenograms must excite surprise unless one has kept pace with the recent advances in roentgenology. Praise must be given the author and the publisher for the beautiful way in which the book is put out. Paper, type, illustrations are excellent. The plates showing roentgenograms, electrocardiograms and pathologic specimens are all that could be asked. Many of these specimens are of hearts that have been hardened and cut in such a manner as to bring out the salient points better than the conventional cuts would have done.

Pathology, Microbiology and Experimental Medicine. Issued by the Chinese Society of Pathology and Microbiology on the occasion of the visit to China of Dr. Eugene L. Opie, professor of pathology, Cornell University Medical College, New York. Chinese Medical Journal supplement III. Paper. Pp. 692, illustrated. Price in United States $4.50. Peiping, China: Chinese Medical Journal, 1940.

The supplement series of the *Chinese Medical Journal* has been established to bring together in one place articles on the results of scientific work in China in the fields of pathology, microbiology, parasitology and experimental medicine. Judged by the names of the authors, all the articles in supplement III, with a few exceptions, are by Chinese investigators. These fifty some articles cannot be reviewed individually. They contain well written, well illustrated and well printed reports of the results of studies on diseases and conditions more or less peculiar to China, which is true especially of those in the field of parasitology, as well as of researches on problems of fundamental significance. As pointed out in the introduction by Dr. Eugene L. Opie, to whom this supplement is dedicated, the articles in it "constitute a significant contribution to medical science." The supplement is of interest to all workers in the fields represented.

The Virus: Life's Enemy. Kenneth M. Smith, F.R.S. Cambridge Library of Modern Science. Cloth. Pp. 176, with 19 illustrations. Price $2. New York: The Macmillan Company, 1940.

This book, which is issued under the auspices of the Cambridge Library of Modern Science, "gives an account of the discovery and pursuit of the viruses." The first part deals with the nature of viruses and describes their discovery, the methods by which they are studied and the current conceptions of their nature. The second part takes up viruses in action, including: "how viruses get about," i. e., the modes of virus infection; the relations between viruses and the insects, which spread them; the results of the growth of viruses in living cells; important virus diseases in man, animals (mammals, birds, fishes, insects), plants and bacteria; prevention and control of virus diseases. An appendix lists important virus diseases. The style is clear and lively. The illustrations are instructive; they show especially well the lesions of plant diseases caused by viruses—"no fewer than 135 separate plant viruses have been described." One has in this book a good summary of a general and popular nature of present knowledge of viruses and virus diseases.